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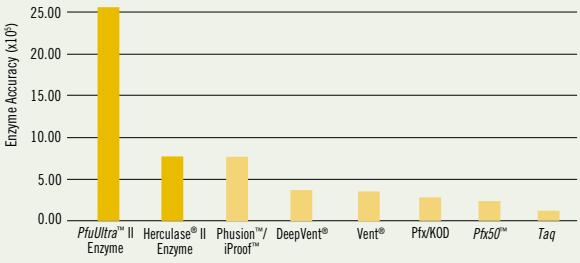

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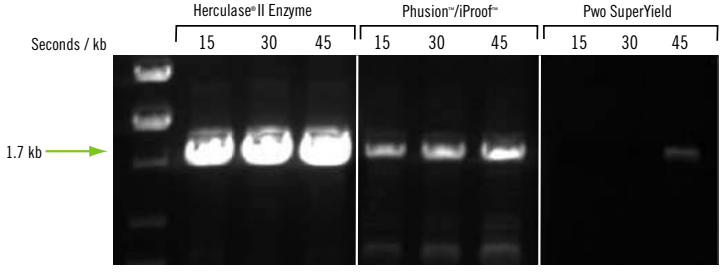

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Volume 311, Issue 5763

COVER

Schematic view of Uranus and its rings and inner moons. Recent Hubble Space Telescope images revealed the wide, tenuous outer two rings and several tiny moons. The planet itself is shown in approximately real color. Small moons are shown as sequences of colored dots that represent their orbital motion. See page 973.

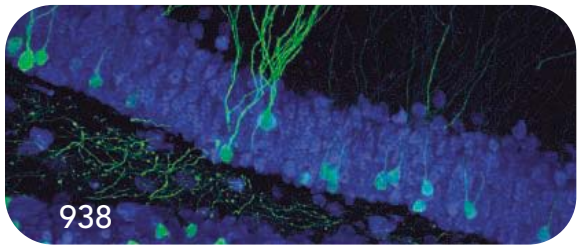
Image: M. R. Showalter

DEPARTMENTS

EDITORIAL

NEWS OF THE WEEK

NEWS FOCUS



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PERSPECTIVES

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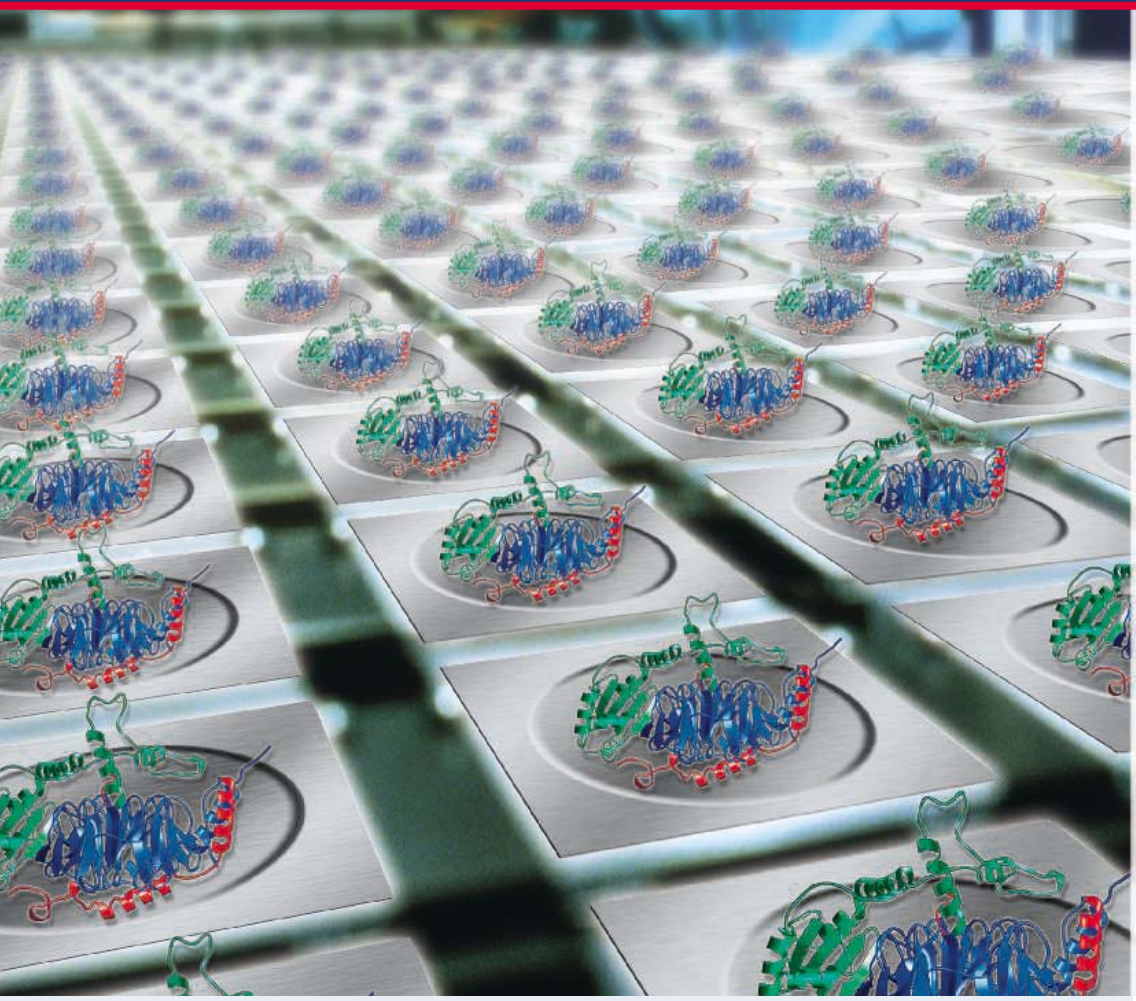
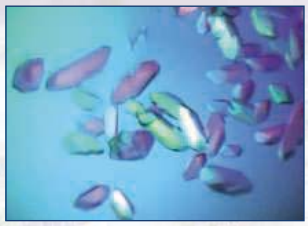
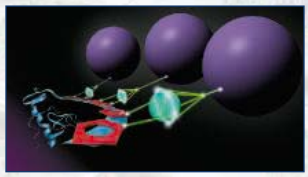
Systems Biology — Proteins and Proteomics

Standardized solutions for proteins





E. coli gyrase A C-terminal domain crystals. Courtesy of Alex Ruthenburg from Prof. Verdine's laboratory, Harvard University, Boston, USA.



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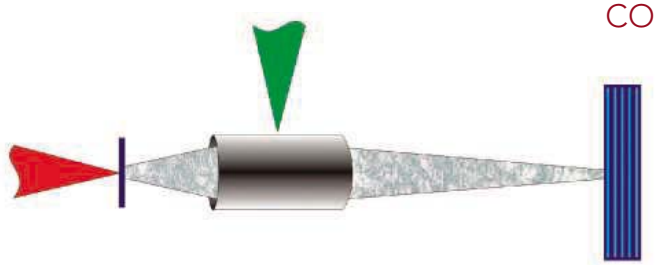


SCIENCE EXPRESS

www.sciencexpress.org

MEDICINE

A Protein Farnesyltransferase Inhibitor Ameliorates Disease in a Mouse Model of Progeria

L. G. Fong, D. Frost, M. Meta, X. Qiao, S. H. Yang, C. Coffinier, S. G. Young

A drug that inhibits addition of lipids to proteins has beneficial effects in a mouse version of a rare premature aging disorder, suggesting that it may be useful in children with the disease.

>> News story p. 934

10.1126/science.1124875

IMMUNOLOGY

Selective Stimulation of T Cell Subsets with Antibody-Cytokine Immune Complexes

O. Boyman, M. Kovar, M. Rubinstein, C. D. Surh, J. Sprent

The paradoxical stimulation of memory immune cells is explained by an unusual activation of a growth factor when bound to an antibody, usually thought to be inhibitory.

10.1126/science.1122927

APPLIED PHYSICS

Ultrafast Laser–Driven Microlens to Focus and Energy-Select Mega–Electron Volt Protons

T. Toncian et al.

A coordinated pair of intense laser pulses—one on a thin solid and one on a small cylinder connected to it—can produce a focused beam of high-energy protons.

10.1126/science.1124412

DEVELOPMENTAL BIOLOGY

Zebrafish MiR-430 Promotes Deadenylation and Clearance of Maternal mRNAs

A. J. Giraldez et al.

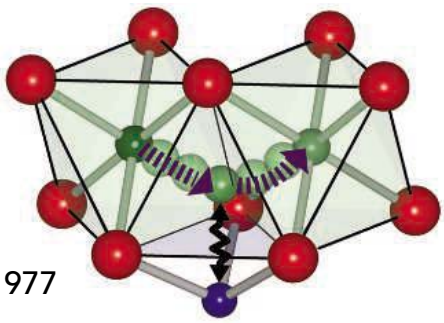
A small regulatory RNA promotes the degradation of the maternal messenger RNAs that are packaged into the oocyte to guide the first steps of animal development.

10.1126/science.1122689

REVIEW

BREVIA

RESEARCH ARTICLE



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REPORTS

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Illuminate the Mystery of Biological Dark Matter

First referred to as the "biological equivalent of dark matter" in the October 26, 2001 issue of *Science**, microRNAs (miRNAs) are small, highly conserved RNA molecules that act as key regulators of development, cell proliferation, differentiation, and cell cycle. miRNAs have been implicated in oncogenesis and viral infection. Explore this emerging field with a complete portfolio of innovative products specifically designed for miRNA investigation.

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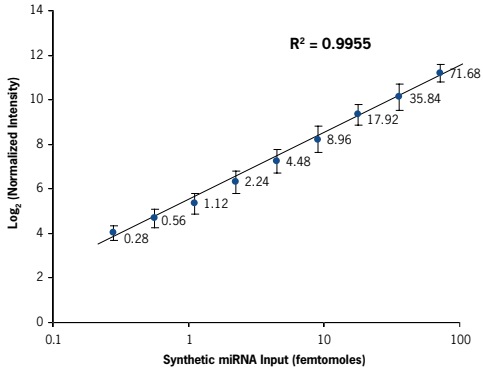

Linearity and Sensitivity of the *mir*Vana™ Bioarray System. Chemically synthesized oligonucleotides corresponding to ten mature miRNA sequences were spiked into FirstChoice® Total RNA samples. The synthetic miRNAs were spiked in at known amounts (0.28–71.68 femtomoles), and arrayed in a Latin Square format. Samples were subjected to the whole *mir*Vana Bioarray process (fractionation, labeling, and hybridization). The graph shows the average of the normalized signal intensities of the ten spiked synthetic miRNAs for each input amount.

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* Ruvkun, G. 2001. Glimpses of a tiny RNA world. *Science* 294(Oct. 26):797-799.

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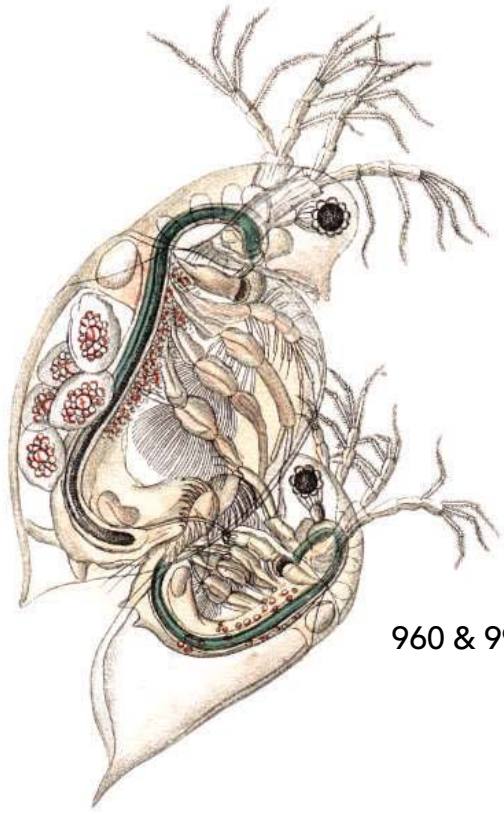

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REPORTS *CONTINUED...*

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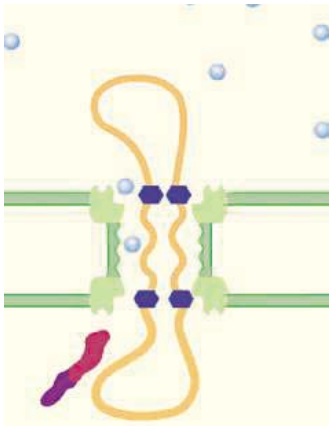

Passing from cell to cell.

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TEACHING RESOURCE: Movement of Macromolecules in Plant Cells Through Plasmodesmata
R. A. Jorgensen and W. J. Lucas
Two animations show how transcription factors can move from cell to cell.

RESOURCES: ST on the Web
Explore the Orientations of Proteins in Membranes database.

The two sides of Cdk5.

SCIENCE'S **SAGE KE**

www.sageke.org SCIENCE OF AGING KNOWLEDGE ENVIRONMENT

PERSPECTIVE: When Good Cdk5 Turns Bad
Q. Guo
Prolonged activation of the cyclin-dependent kinase-5 leads to neurodegeneration.

NEWS FOCUS: Mucking With Metabolism
M. Beckman
Inability to repair DNA damage in mitochondria could foster metabolic syndrome.

SCIENCE**NOW**

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Ozone "Recovery" May Be Solar Trick
Intense radiation from the sun, not CFC ban, could account for increased ozone.

Who Needs Dark Energy?
Modified gravity might explain the accelerating expansion of the universe.

Tracing HIV's Steps
Genetic analyses fill in important steps between monkey and man.

Giving your new business a lift.

SCIENCE **CAREERS**

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

US: Tooling Up—Crises and Career Stages, Part 2
D. Jensen
Sometimes it takes a crisis to break out of the inevitable career "plateaus."

EUROPE: Scientific Entrepreneurship—Getting a New Business Off the Ground
E. Pain
Aeronautics engineer Françoise Heilmann-Pascal started a company that rents dirigibles for research and tourism.

MISCINET: Social and Behavioral Sciences—Finding Solutions to Society's Ills
R. Arnette
Social psychologist Monique Clinton-Sherrod studies domestic violence and substance abuse.

MISCINET: Same School, Different Program, All Part of the Plan
C. Parks
Cherie Butts talks about her transition from undergraduate to graduate school at Johns Hopkins University.

US: Profile—Peter Lu
J. Austin
When it comes to science, Harvard graduate student Peter Lu just wants to have fun.
>> News story p. 931

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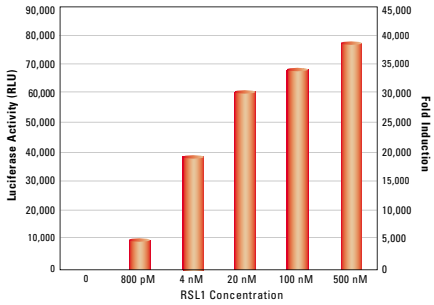

Luciferase expression in response to increasing RSL1 concentrations. NIH3T3 cells co-transfected with pNEBR-R1 and pNEBR-X1 expressing firefly luciferase were induced with the indicated RSL1 concentrations. Expression was measured 48 hours post-induction.

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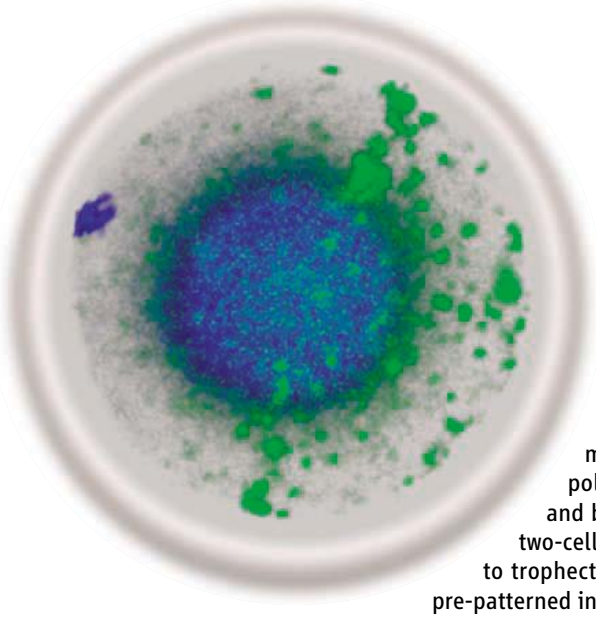


Knowing Your Head from Your Toes

Embryos of various organisms, such as insects and amphibians, establish head and tail ends at a very early stage because of the localization of cell fate determinants during oogenesis. Mammalian embryos have been thought to be different, with equipotent blastomeres in the early stages. **Deb *et al.*** (p. 992) show that early mouse embryos may also have localized determinants. In particular, *Cdx2* messenger RNA is asymmetrically localized toward the vegetal pole of mouse oocytes, changes orientation after fertilization, and becomes concentrated in the late dividing blastomere of the two-cell-stage embryo. Thereafter, it marks the cell lineage leading to trophectoderm. Thus, specification of the trophectoderm is already pre-patterned in the mouse oocyte.

Messy Moon Motions

Two additional moons, named Mab and Cupid, and two outer rings have been discovered around Uranus by **Showalter and Lissauer** (p. 973, published online 22 December 2005; see the cover and the Perspective by **Murray**). These new members of the uranian system were spotted in images from the Hubble Space Telescope and traced in earlier pictures from Voyager 2. Substantial changes are seen in the passages of the moons and brightness of the rings since the Voyager 2 fly-by. Many of Uranus' moons do not follow simple keplerian orbits but exhibit complex dynamics, which suggest that the whole system is gravitationally unstable or chaotic.

Martian Aurorae

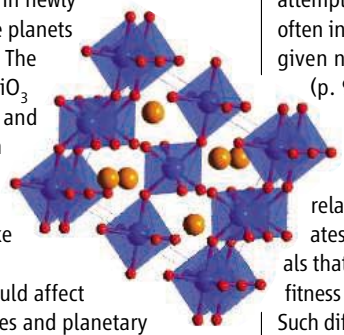
Aurorae occur when charged particles are accelerated along magnetic field lines into a planetary atmosphere. **Lundin *et al.*** (p. 980) have mapped the motions of ions and electrons flowing in arcs above Mars using the ASPERA-3 experiment on board the orbiting Mars Express spacecraft. The looped paths of charged particles in the martian atmosphere are associated with regions of strong magnetism on the planet's surface, where aurorae have also been seen. This formation mechanism for aurorae on Mars is similar to the one for Earth.

Power to the People Movers

Despite their high energy density, lithium batteries are not used in cars and other transportation applications because they cannot deliver power at a sufficiently high rate. **Kang *et al.*** (p. 977) report a combined theoretical and experimental exploration of a class of battery electrodes with a layered transition-metal structure that permits much faster lithium ion transport. The results suggest a general strategy for improving lithium-battery power delivery.

Metallic Mantle Minerals

In smaller terrestrial planets having an iron core, the main silicate mineral at depth is thought to be composed of $MgSiO_3$, but its stability at higher pressures cannot yet be determined experimentally. **Umemoto *et al.*** (p. 983) used numerical calculations to infer its stability at extreme conditions that may be obtained in the giant outer planets or in newly found, large Earth-like planets in other solar systems. The results imply that $MgSiO_3$ will dissociate to MgO and SiO_2. The compression of electronic orbitals at high pressure will lead to more metal-like behavior of these compounds, which would affect their thermal properties and planetary heat flow.



Going Faster

How much meltwater the Greenland Ice Sheet may be contributing to global sea-level rise depends on the mass balance between the interior of the ice sheet and its margins. The present understanding is that the interior is gaining mass but the margins are eroding even more rapidly. **Rignot and Kanagaratnam** (p. 986; see the Perspective by **Dowdeswell**) present an ice velocity map of the entire Greenland Ice Sheet and estimate the rate of ice discharge around its entire margin. A comparison of their results to past data shows that there has been a widespread acceleration of ice flow since 1996, that mass loss has doubled in that time, and that ice dynamics, which are particularly dependent on warming, dominate the rapid retreat of Greenland's glaciers.

Rethinking Sexual Selection

Much that Darwin said about sexual selection in 1871 is culturally and socially biased. His theory attempts to explain why males and females differ, often in ways that are contrary to expectations given natural selection. **Roughgarden *et al.*** (p. 965) offers an alternative model that presents social selection theory based on cooperative game theory. Thus, cooperation among individuals in sexual relations, as in other social relations, generates advantages such that groups of individuals that succeed in cooperation may have greater fitness vis-à-vis groups that fail to cooperate. Such differences could generate selection pressure toward individuals and groups that cooperate.

Sex Pays Off

Sex is expensive. For example, the daughters of an asexual female can reproduce at twice the rate of the progeny descended from a sexual female, assuming a sex ratio of one male to one female. So why is sex maintained despite this apparent disadvantage? One suggestion has been that the lack of meiotic recombination in asexual lineages results in the accumulation of mutations in asexuals. **Paland and Lynch** (p. 990; see the Perspective by **Nielsen**) studied sexual and obligate

Continued on page 915

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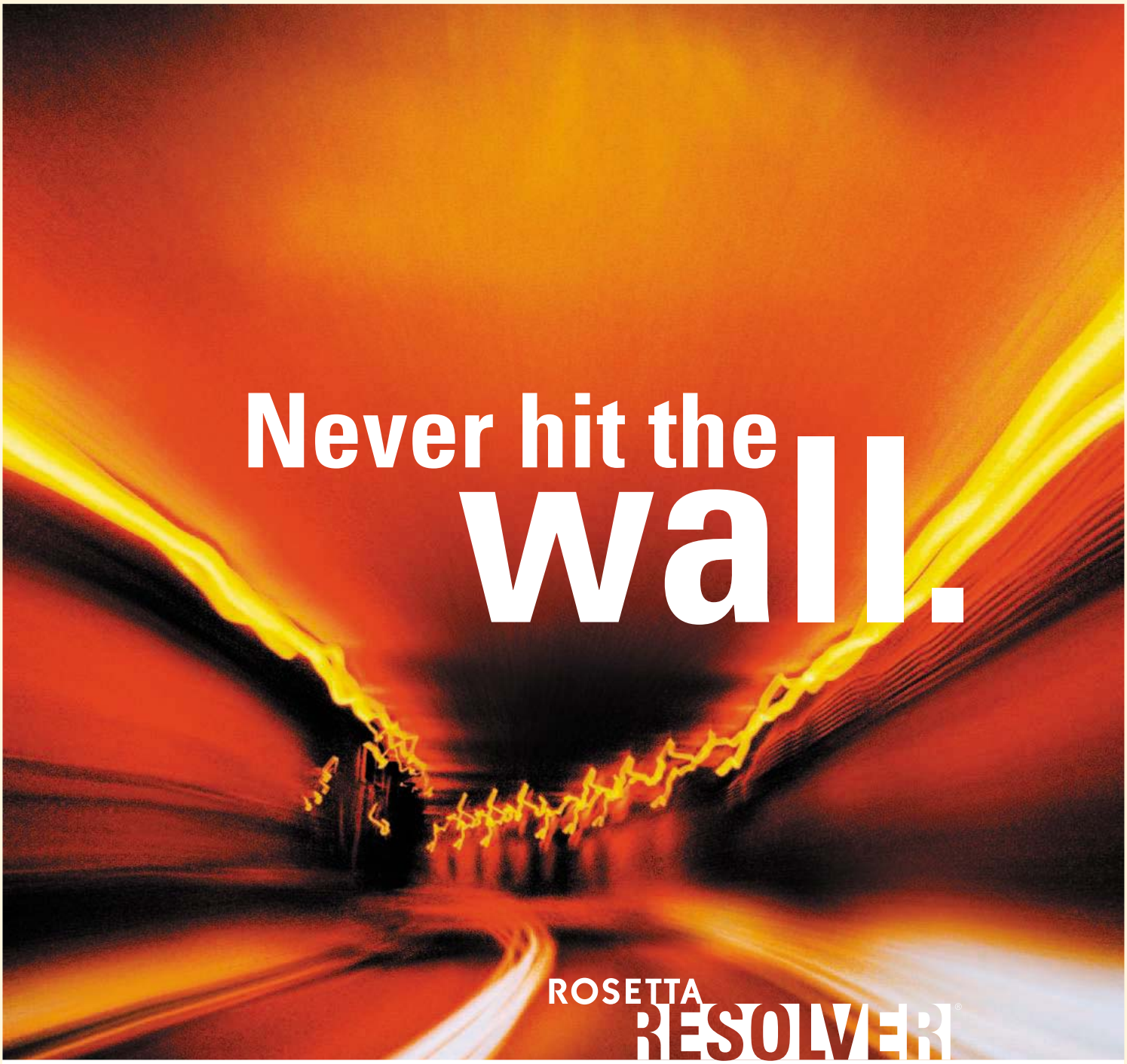

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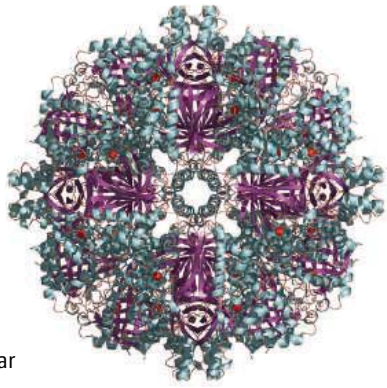
Continued from page 913

asexual lineages of *Daphnia* (water fleas). Through a process of selective interference, the asexual lineages developed a fourfold greater number of mildly deleterious mutations in their mitochondrial genomes compared to the sexual lineages.

Microbial Mobilization of Elemental Sulfur



Microbial oxidation of elemental sulfur is important in the global sulfur cycle, but little is known about the mechanism of this reaction. **Urich *et al.*** (p. 996) have determined a 1.7 angstrom resolution structure of a sulfur oxygenase reductase from a thermoacidophilic archaeon. A spherical, positively charged reaction chamber forms from 24 monomers. Linear sulfur probably enters through apolar channels and is bound by a cysteine persulfide in one of the 24 active sites. This sulfane sulfur chain is the substrate of disproportionation and oxygenation at a nearby mononuclear nonheme iron.

Revving Up the Circadian Clock

In mammals, circadian rhythms regulate many aspects of behavior and physiology, including sleep-wake cycles and metabolism. Disruption of these rhythms is associated with certain psychiatric illnesses such as bipolar disorder. **Yin *et al.*** (p. 1002) describe a potential molecular link between circadian clock control and bipolar disorder. In cultured fibroblasts, a key negative regulator of clock gene expression, the Rev-erbα nuclear receptor, was rapidly degraded after exposure to lithium, which is used in treating bipolar disorder. This destabilization of Rev-erbα led to activation of clock genes.

Don't Think Too Much

We hope that thinking about a decision results in a good choice, and that the more complex the decision, the more time and effort were invested in thinking about it. **Dijksterhuis *et al.*** (p. 1005; see the news story by **Miller**) show that deliberate thinking about simple decisions (such as buying a shampoo) does yield choices that are judged to be more satisfying than those made with little thought, as expected. However, as the decisions become complex (more expensive items with many characteristics, such as cars), better decisions and happier ones come from not attending to the choices but allowing one's unconscious to sift through the many permutations for the optimal combination.

Norepinephrine, Pleasure, and Reward

Although norepinephrine is generally accepted to play a role in the adverse effects of opiate withdrawal, its role in mediating the rewarding and stimulatory effects of opiates remains controversial. **Olson *et al.*** (p. 1017) discovered that genetically engineered mice unable to synthesize norepinephrine, due to a targeted disruption of the dopamine β-hydroxylase (DBH) gene, appear totally blind to morphine reward, as measured in a conditioned place preference test. Importantly, sensitivity to morphine reward was completely rescued by restoration of DBH expression in a specific set of neurons.

Rats Are Smarter Than We Think

Although both human and nonhuman animals may use basic associative mechanisms to learn about causal relations, humans have a deeper understanding of causal relations that cannot be reduced to associative learning. In contrast, there is no definite proof that animals, including nonhuman primates, possess deep causal understanding. **Blaisdell *et al.*** (p. 1020) present evidence that rats can reason about the effects of their causal interventions. Rats correctly predicted that interventions on one effect of a common-cause model would not affect the other effect. Thus, rats can engage in more sophisticated causal reasoning than predicted by associative models.

CREDIT: URICH *ET AL.*

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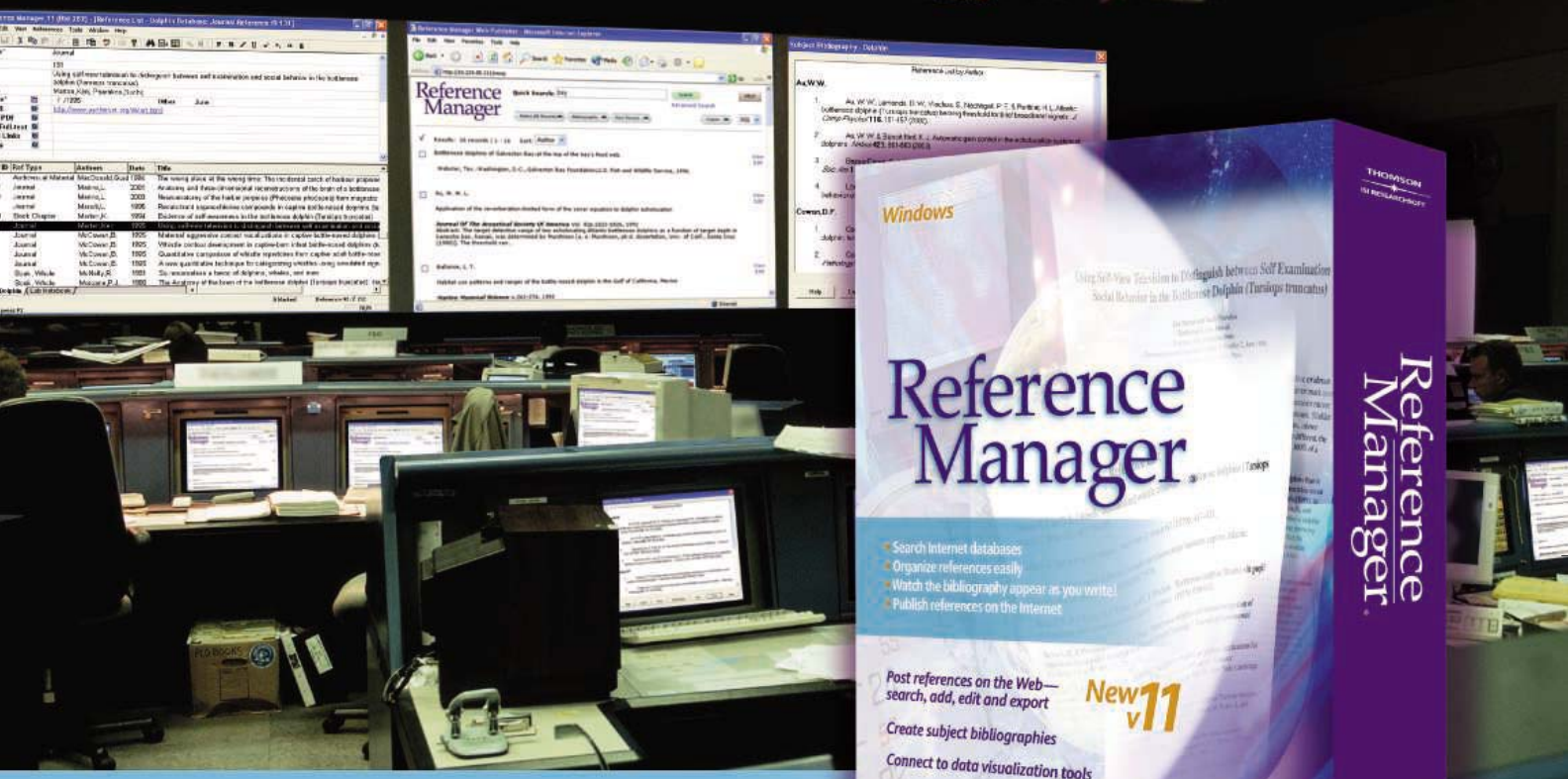

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Donald Kennedy is Editor-in-Chief of *Science*.

The New Gag Rules

THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION (NASA) AND THE NATIONAL OCEANIC AND Atmospheric Administration (NOAA) are among the most popular and scientifically sophisticated agencies in the U.S. government. Not only do they do good science, they do dramatic, risky, and even romantic things—capturing comet dust, sending surveyors to Mars, flying airplanes into hurricanes, and providing images of impending weather events. They are full of productive, respected scientists. We have published papers from groups at both agencies and have been proud to do so.

But these days, we're trying to figure out what is happening to serious science at NOAA and NASA. In this space a month ago, I described some of the research that supports a relationship between hurricane intensity and increased water temperatures. Two empirical studies, one published in *Science* and one in *Nature*, show that hurricane intensity has increased with oceanic surface temperatures over the past 30 years. The physics of hurricane intensity growth, worked out by Kerry Emanuel at the Massachusetts Institute of Technology, has clarified and explained the thermodynamic basis for these observations.

Yet a NOAA Web site* denies any relationship between global climate change and hurricane strength. It attributes the latter instead to "tropical multidecadal signals" affecting climate variability. Emanuel has tested this relationship and presented convincing evidence against it in recent seminars. As for the many NOAA scientists who may agree with Emanuel, the U.S. Department of Commerce (the executive agency that NOAA is part of) has ordered them not to speak to reporters or present papers at meetings without departmental review and approval.

That's bad enough, but it turns out that things are even worse at NASA, where a striking front-page story by Andy Revkin in the *New York Times* (28 January 2006) details the agency's efforts to put a gag on James Hansen, director of the agency's Goddard Institute for Space Studies, after a talk he gave at a meeting of the American Geophysical Union in San Francisco in December 2005. His sin was that he pointed out that the climate change signal is now so strong, 2005 having been the warmest year in the past century, that the voluntary measures proposed by the administration are likely to be inadequate.

Hansen was told that there would be "dire consequences" if such statements continued. The *Times* story identifies two NASA public affairs officials, Dean Acosta and George Deutsch, as responsible for delivering this news and insisting that Hansen's "supervisors" would have to stand in for him at public appearances. Those will presumably take place in approvable venues and certainly not on National Public Radio (NPR). Deutsch is reported to have rejected a Hansen interview requested by NPR on the grounds that it was "the most liberal news outlet in the country."

For at least two reasons, this event may establish a new high-water mark for bureaucratic stupidity. First, Hansen's views on this general subject have long been widely available; he thinks climate change is due to anthropogenic sources, and he's discouraged that we're not doing more about it. For NASA to lock the stable door when this horse has been out on the range for years is just silly. Second, Hansen's history shows that he just won't be intimidated, and he has predictably told the *Times* that he will ignore the restrictions. The efforts by Acosta and Deutsch are reminiscent of the slapstick antics of Curley and Moe: a couple of guys stumbling off to gag someone who the audience knows will rip the gag right off.

These two incidents are part of a troublesome pattern to which the Bush administration has become addicted: Ignore evidence if it doesn't favor the preferred policy outcome. Above all, don't let the public get an idea that scientists inside government disagree with the party line. The new gag rules support the new Bush mantra, an interesting inversion of Secretary of Defense Donald Rumsfield's view on war: "You don't make policy with the science you have. You make policy with the science you WANT." But the late-breaking good news is that NASA Administrator Griffin has said that there will be no more of this nonsense, and Deutsch, the 24-year-old Bush appointee sent to muzzle Hansen, has left the agency abruptly after his résumé turned out to be falsified. A change of heart? Stay tuned.

–Donald Kennedy

10.1126/science.1125749

*www.magazine.noaa.gov/stories/mag184.htm

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HIGHLIGHTS OF THE RECENT LITERATURE

PSYCHOLOGY

Unintentional Music Sharing

A window into our souls.

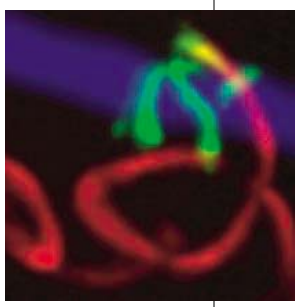
Might our selves be revealed by our choices in music? Rentfrow and Gosling explored this question by asking 74 college students to provide individual top-10 lists of their favorite songs, which were then recorded onto CDs. The students were also asked to provide self-report ratings on personality measures, such as extraversion and conscientiousness; terminal and instrumental values, such as a comfortable life and ambition; and affect and self-esteem. Eight listeners were then asked to rate the students on the same criteria, solely on the basis of hearing their music selections. The measures for which listener judgments correlated most strongly with the self-report data were the personality trait of openness to experience and the instrumental value of imagination. Furthermore, three other listeners had previously coded the songs for 25 experimentally tested musical attributes (for instance, the amount of singing), and these characteristics also displayed correlations with openness and imagination (along with several other traits and values). The results show a differentiating and consistent linkage between our musical tastes and the impressions of us that strangers form purely from learning which songs we like. — GJC

Psychol. Sci. **17**, 236 (2006).

MICROBIOLOGY

Secret Life Exposed

The parasites that cause malaria, *Plasmodium* spp., have been caught on video during a previously hidden portion of their life cycle. Amino *et al.* used epifluorescence time-lapse microscopy to track parasites engineered to express green fluorescent protein as they wended their way through hairless mice. The parasites were injected into mouse skin as sporozoites by a mosquito, and although many traced a path into blood vessels, a significant proportion either actively invaded lymph vessels or remained in the skin. Sporozoites in the lymph system were previously thought to drain into the blood, but in this study, most were shown to be captured in proximal lymph glands. Interestingly, sporozoites injected by syringe instead of mosquito proved 20 times less likely to invade the lymph ducts. The parasites in the lymph node partially transformed into exoerythrocytic forms (EEFs) within the host's dendritic cells and subsequently appeared to degrade completely. Simultaneously, their sister sporozoites that reached the liver through the blood developed normally. Presumably, the degrading EEFs in the dendritic cells deliver EEF-stage antigens, which may induce tolerance in the host, an important consideration for vaccination strategies that use attenuated sporozoites. — CA



Time-lapse image (red to green to yellow) of the sporozoite invading a blood vessel (blue).

Nat. Med. **12**, 220 (2006).

ECOLOGY/EVOLUTION

Eggs on the Rise

A bird's clutch size—the quantity of eggs laid during a nesting period—is a central feature of a bird's life history, but has presented an evolutionary conundrum. Although studies of bird species have predicted the existence of positive selection for increasing clutch size over time, such increases have failed to materialize during long-term observation, perhaps because of constraints imposed by correlated environmental factors that also affect fitness.

In a 25-year study of mute swans, Charmantier *et al.* observed not only the expected directional selection for increasing clutch size, but also an actual increase, of 0.35 standard deviations, across the population. Reduced predation and increased food supply over the course of the study may have fostered the increase. Because the authors kept track of the pedigrees of all of the individuals in the study, they garnered strong evidence that these changes were genetic rather than phenotypic, and hence that a clear microevolutionary change took place over the course of a quarter century. — AMS

Am. Nat. **167,** 10.1086/499378 (2006).

CELL BIOLOGY

Perfect Packaging

Endothelial cells that line the blood vessels are packed with cigar-shaped organelles termed Weibel-Palade bodies. These secretory storage granules are filled with a protein known as von Willebrand's factor (VWF), which, when released from the cell, plays a key role in reestablishing the integrity of damaged blood vessels by recruiting platelets to the site of injury. Michaux *et al.* found that low pH within the storage granule is important to generate and maintain the tubular folding of the VWF, which in turn defines the morphology of the granule. Thus, the folding of VWF into tubules generates the unique architecture of the Weibel-Palade bodies.

The authors further sought to learn if this well-defined geometry has a functional significance beyond packaging and storage. They found that the tubular packaging is important during secretion to allow the VWF to unfold rapidly and efficiently into very long fibrils—up to 100 times the

Continued on page 921

CREDITS (TOP TO BOTTOM): JOSHUA MOGLIA/*SCIENCE*; AMINO *ET AL.*, *NAT. MED.* **12**, 220 (2006)

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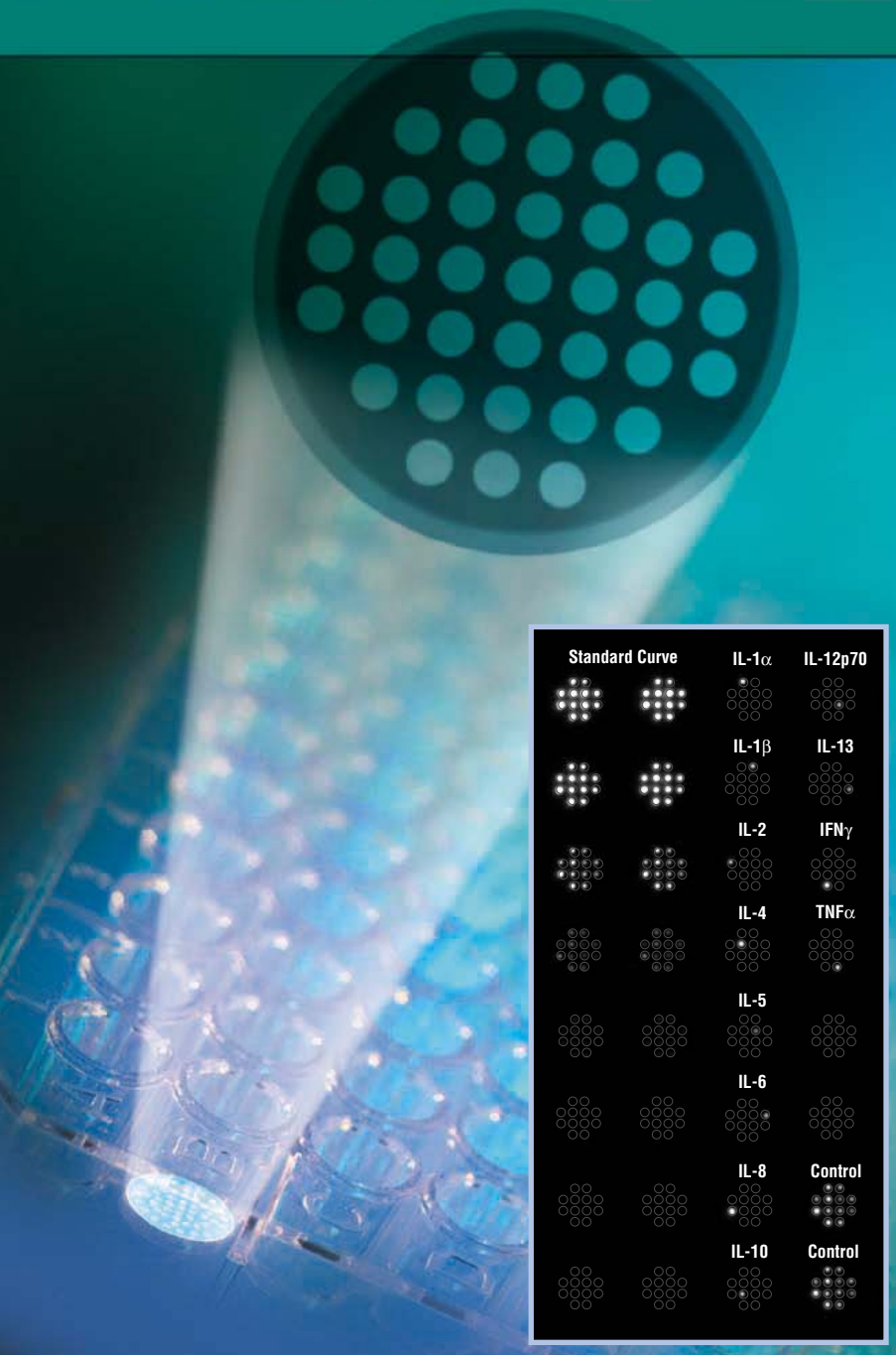

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PERBIO

Continued from page 919

length of the packaged protein tubules—in order to trap circulating platelets. If folding is aberrant, or if a rise in granule pH interferes with packaging, VWF fails to unfold fully—presumably due to premature unraveling and tangling of the polypeptide before secretion—and platelet capture is severely compromised. — SMH

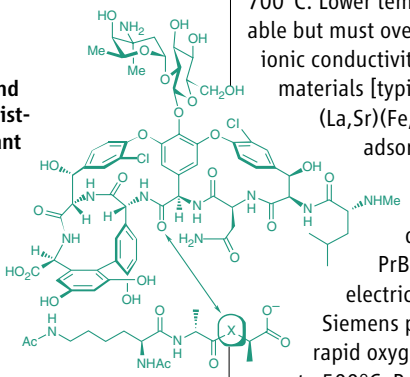
Dev. Cell **10**, 223 (2006).

CHEMISTRY

Restored Affinity

Vancomycin is a powerful antibiotic, which functions by binding to a pair of alanine residues and thereby disrupting the formation of bacterial cell walls. However, several strains of bacteria can evolve to resist vancomycin through replacement of the terminal alanine with lactate. This structural substitution of an O atom for an N-H group reduces vancomycin binding affinity by a factor of 1000.

In a preliminary effort to combat this resistance pathway, Crowley and Boger have modified the vancomycin structure. Their prior modeling studies attributed the reduced affinity to lone pair repulsion between the lactate oxygen and a carbonyl oxygen in the vancomycin framework. They therefore prepared a synthetic derivative with a methylene group replacing the offending carbonyl. This backbone substitution was deemed too fundamental a change to attempt by modifying intact vancomycin. Instead, the authors were able to adapt their prior total synthesis of the native compound by introducing the methylene group at the outset and protecting the adjacent nitrogen as a carbamate. The resulting compound showed a 40-fold improvement in activity against cultures of resistant bacteria, with only a 37-fold loss in affinity toward the Ala-Ala motif present in nonresistant strains. — JSY



Vancomycin structure and binding motif in nonresistant (X = NH) and resistant (X = O) bacteria.

CREDIT: CROWLEY AND BOGER, *J. AM. CHEM. SOC.* 10.1021/JA0572912 (2006)

J. Am. Chem. Soc. 10.1021/ja0572912 (2006).

ASTRONOMY

Seeking Planets in the Dust

To understand planet formation in our solar system and beyond, astronomers search for dusty debris disks around stars like the Sun. Kalas *et al.* have spotted light scattered by low-mass disks around two stars that are close to a billion years old. In order to make these observations, the authors used the sensitive Advanced Camera for Surveys on board the Hubble Space Telescope; an inserted coronagraph mask permitted a clear field of view by blocking the stars' central glare.

The two disks have different shapes, due to distinct inclination and intrinsically different architectures. One appears as a narrow belt of dust, concentrated 83 astronomical units (AU) from the star, with an outer edge truncated abruptly at 109 AU. In contrast, the other star's disk extends out to 110 AU without significant narrowing, despite the old age of the star.

On the basis of these characteristics and those observed in similar studies, the authors propose two limiting classes of disk morphology: narrow belts and wide disks. The former could arise from early stochastic dynamical events that expel material and heat the disk, with nascent planets sweeping up the dust at certain radii, perhaps mirroring the early stages of our own solar system. The absence of these features in the wide disk morphology suggests that planet formation may not be ubiquitous in dust clouds. — JB

Astrophys. J. **637**, L57 (2006).

MATERIALS SCIENCE

Cooler Running

Current solid-oxide fuel cells run at 500° to 700°C. Lower temperature operation is desirable but must overcome low electronic and ionic conductivity in the ceramic cathode materials [typically $(La,Sr)MnO_3$ or $(La,Sr)(Fe,Co)O_3$] where oxygen is adsorbed and reduced to oxide. Kim *et al.* have found that the oxygen-deficient double-perovskite material $PrBaCo_2O_{5+\delta}$ (PBCO) has high electrical conductivity (~100 Siemens per square centimeter) and rapid oxygen transport kinetics at 300° to 500°C. Prior screening for improved cathodes has generally assessed candidate materials in porous bulk morphologies. To achieve a more precisely ordered microstructure, the authors prepared the PBCO as an epitaxial thin film, which was grown on strontium titanate by pulsed laser deposition. They speculate that the increase in oxygen surface exchange rate relative to that of disordered perovskites may arise from the alignment of the PBCO *c* axis in the film plane, which raises the concentration of vacancies into which oxide can diffuse. — PDS

Appl. Phys. Lett. **88**, 024103 (2006).

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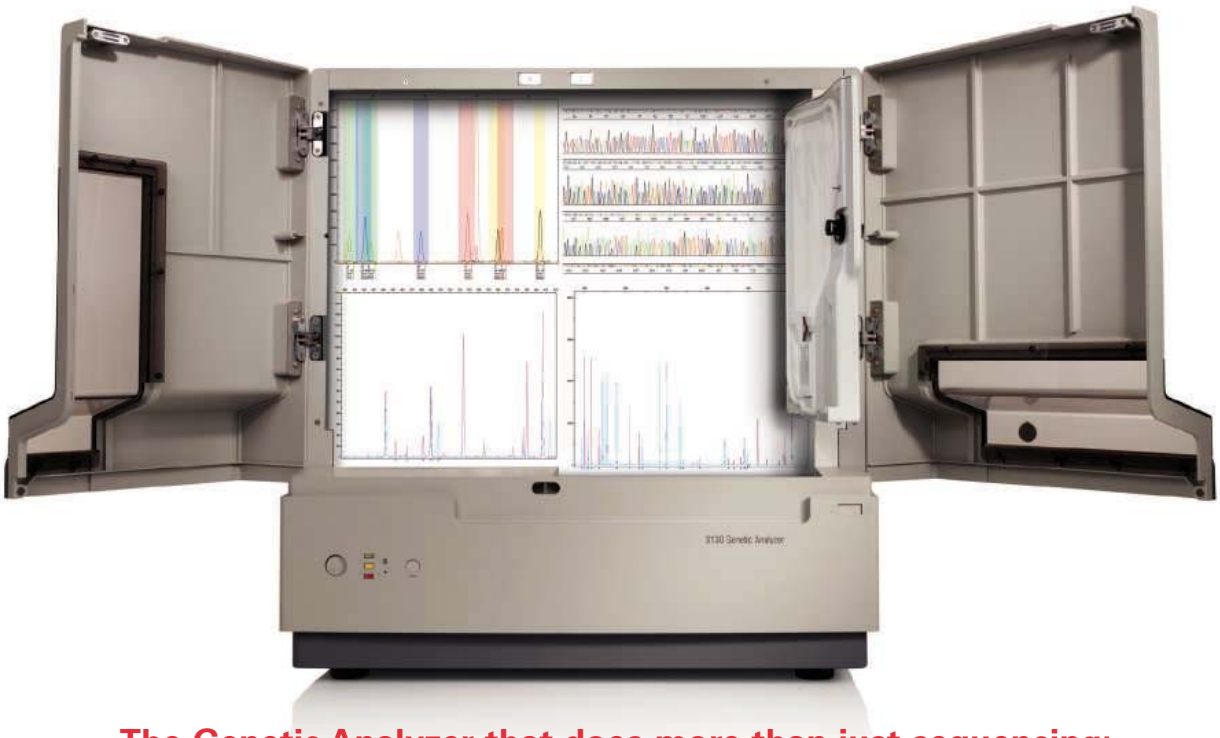

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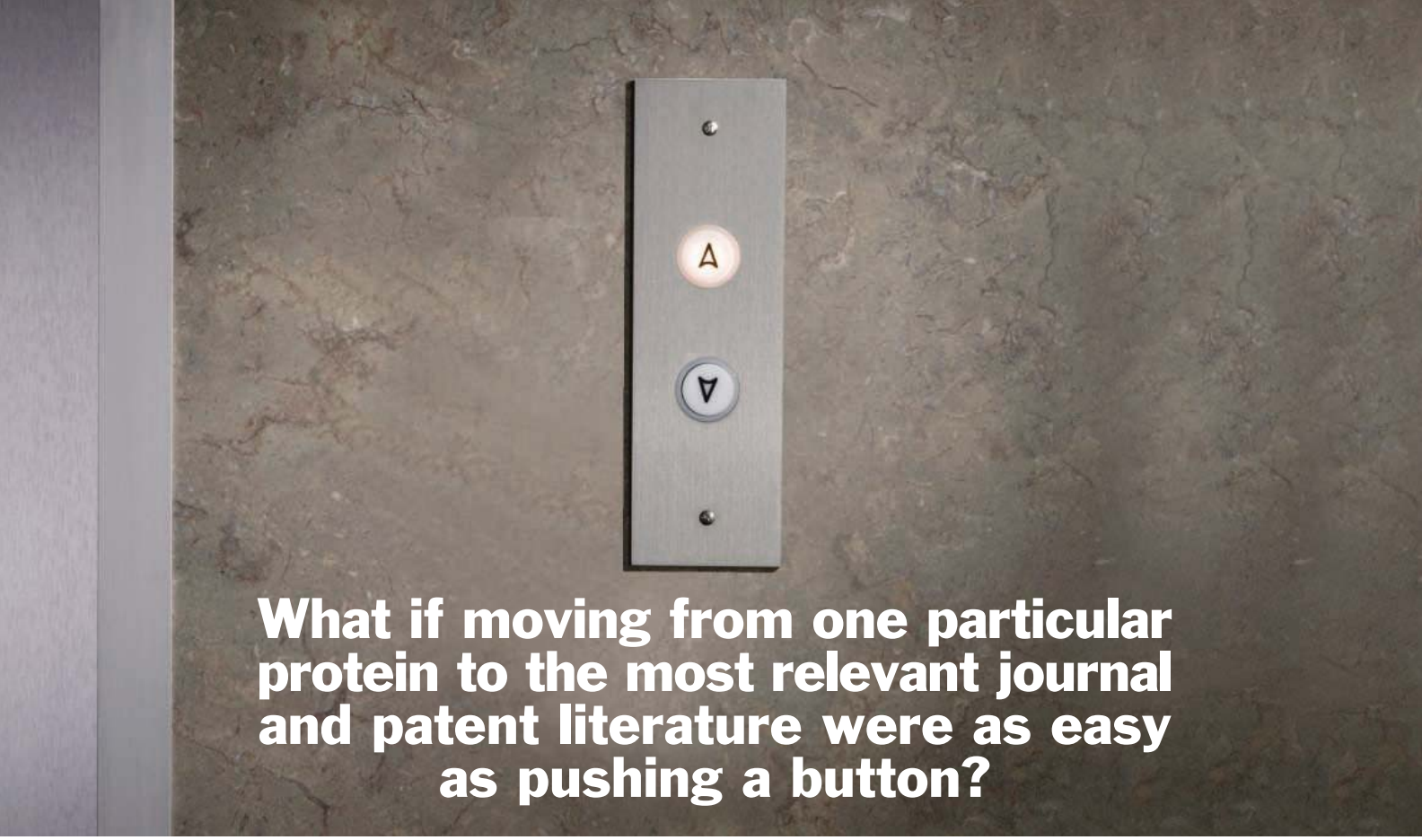

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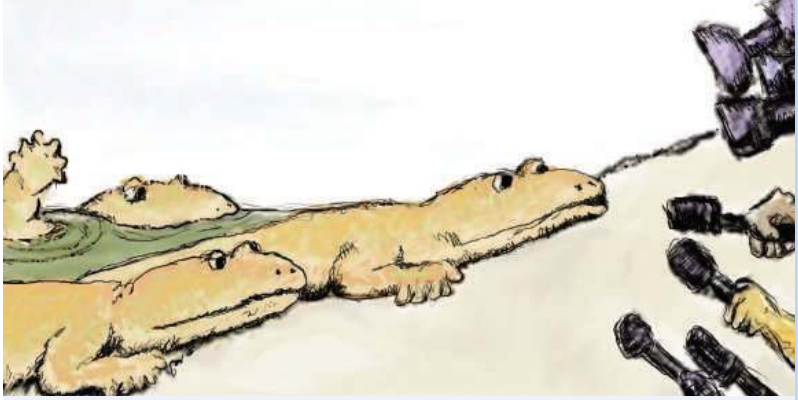


RESOURCES

Fish Stories

With their ferocious dinosaurs and mass extinctions, the Jurassic and the Cretaceous periods attract plenty of attention. But there's a lot to like about the earlier, lesser known Devonian period. The "Age of Fishes" saw major changes in aquatic animals, including the evolution of lobe-finned and ray-finned fishes and the definitive emergence of sharks. To bone up on this epoch, which spanned from 410 million to 356 million years ago, check out Devonian Times.

Webmaster Dennis Murphy, a computer exhibit designer in Pennsylvania, began the newspaperlike site in 1997 with support from researchers at the Academy of Natural Sciences in Philadelphia. Visitors will find basic background on the plants, animals, and geology of the period. And for Devonian diehards, there is a Who's Who of fossil organisms from Red Hill, an important Devonian site in Pennsylvania. A new section describes a humerus found there (*Science*, 2 April 2004, p. 90). Paleontologists believe the arm bone belonged to a limbed fish that may have led the procession of animals transitioning from life in the sea to walking on the ground. Above, Murphy's take on the discovery. >> www.devoniantimes.org

SOUNDS

<< In Tune With the Animals

Wondering what a zebra or a silkworm sounds like? Check out Listen to Nature, which holds 400 samples from the British Library's vast sound collection. Hear clips including a yipping Arctic fox, the chirps of a Namibian sand gecko, and the dawn chorus of creatures in an Australian rainforest.

In The Language of Birds, the site's creators have scattered bird recordings within a review article packed with facts about bird communication. You can listen to a marsh warbler, which steals from other birds' songs, or Alex, an African gray parrot who can reportedly identify colors and objects. Above, a sedge warbler, which stops singing when it finds a mate. >> www.bl.uk/listentonature

EDUCATION

Professor's Assistant

If you're a physicist or astronomer who's rounding up Web materials for a course, save some time by visiting ComPADRE. This hub for physics and astronomy teaching resources leads to a half-dozen subsites stocked with growing collections of links reviewed by experts. For instance, a search on "black holes" at The Astronomy Center produced 24 hits, including animations and a cosmology primer. The project's sponsors include the American Institute of Physics, the American Astronomical Society, and the American Association of Physics Teachers. >> www.compadre.org

DATABASE

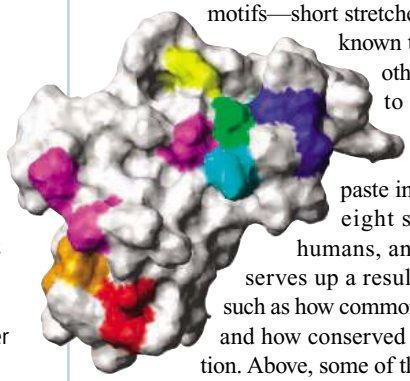
Satellite Tally

Need a complete list of the satellites hovering above Earth? The Union of Concerned Scientists' global security section has toted up all 800 or so active satellites and posted the information as an Excel spreadsheet. Twenty-one data fields include altitude, launch date, manufacturer, and whether the craft is up there for military or civilian purposes. The data show that the United States has the most satellites, followed by Russia and China. >> www.ucsusa.org/satellite_database

TOOLS

Matchmaker

Biologists puzzling over the role of a protein can get help at Minimotif Miner, created by Sanguthevar Rajasekaran, Martin Schiller, and others at the University of Connecticut, Storrs. The new site searches your protein for hundreds of motifs—short stretches of amino acids—that are known to perform specific roles in other proteins, such as binding to or modifying other molecules. Enter the ReqSeq number for your protein or paste in its sequence, choose from eight species (including yeast, humans, and fruit flies), and the site serves up a results page that includes data such as how common the motif is in that species and how conserved it has been through evolution. Above, some of the 39 motifs that Minimotif Miner found in the human prion protein, including two motifs that overlap with mutations (yellow, green) that lead to Creutzfeldt-Jakob disease. >> mnm.engr.uconn.edu



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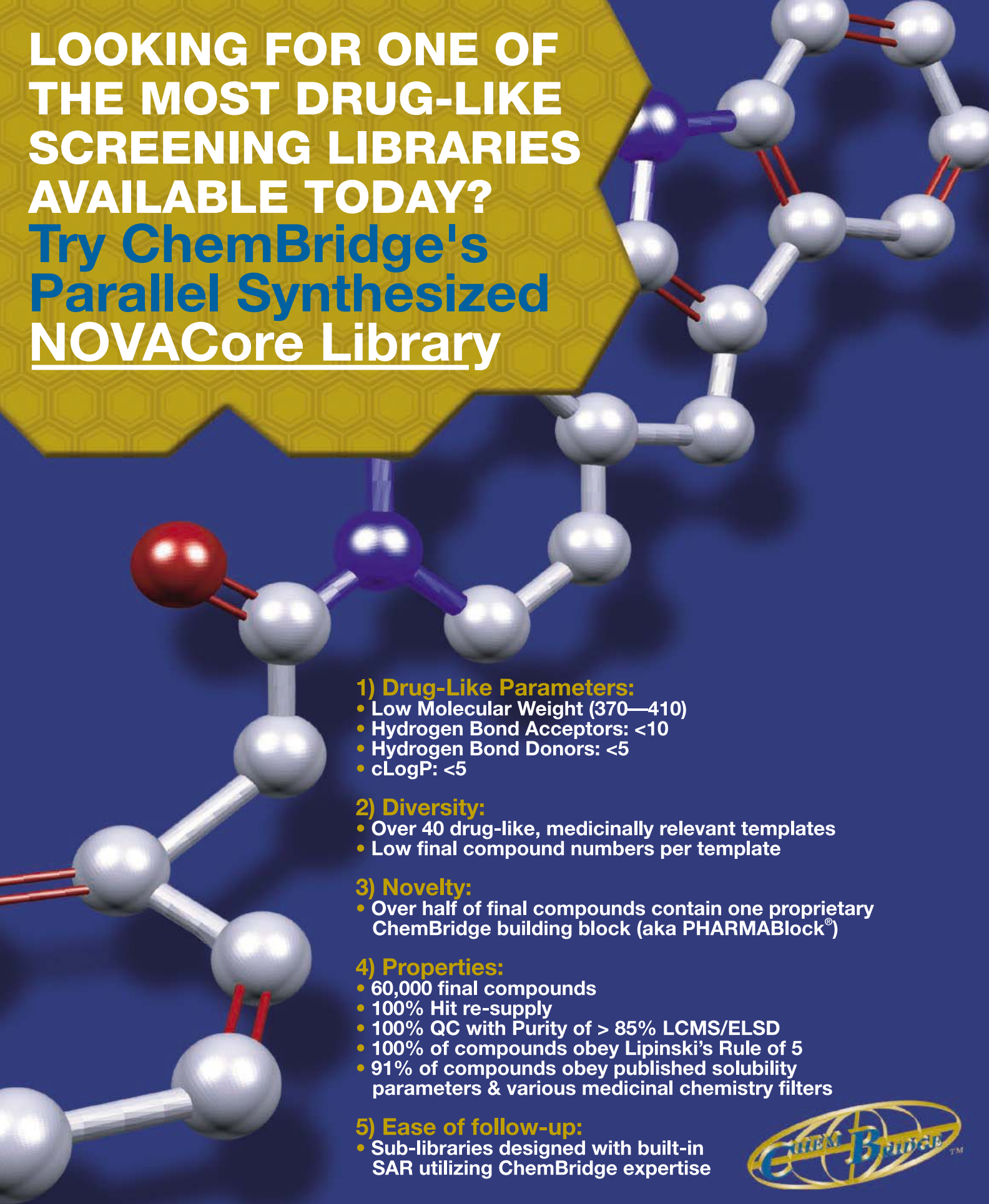

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EDITED BY CONSTANCE HOLDEN

EMPTY SUIT

Although it sounds like a high-tech fraternity prank, "SuitSat" is for real. On 3 February, two astronauts on board the international space station released an old Russian cosmonaut suit loaded with batteries, temperature and power sensors, and radio equipment into the ether.

The mission, sponsored by two space-buff groups, Amateur Radio on the International Space Station and the Radio Amateur Satellite Corp. is to test the durability of the spacesuit and batteries. The public has been encouraged to help track SuitSat's orbit, and reports have been pouring in. Early data suggest that the strength of SuitSat's signal rises and falls as it turns cartwheels in space. The batteries powering the transmitter were expected to last about 120 hours, but the suit will orbit for up to 2 months before burning up in Earth's atmosphere. Aspiring SuitSat trackers can tune FM radios to 145.99 MHz, or go to www.suitsat.org.

CIVIL WAR PTSD

The weapons were different back then, and battlefield medicine has been revolutionized, but scientists studying medical records from the U.S. Civil War of 1860–65 say long-term effects of war on veterans are much the same. Roxane Cohen Silver and colleagues at the University of California, Irvine, have identified more illness, both mental and physical, among Civil War veterans who were exposed to the greatest war trauma.

The researchers matched military records from 15,027 Union Army soldiers with subsequent pension and health records. In the February *Archives of General Psychiatry*, they report that 44% of the men reported signs of mental or "nervous" disease after the war, something that was called "irritable heart" by 19th century physicians. "There are a few detractors that say that PTSD [posttraumatic stress disorder] does not exist or has been exaggerated," says Joseph Boscarino, senior investigator at Geisinger Health System in Danville, Pennsylvania. "Studies such as these are making it difficult to ignore the long-term effects of war-related psychological trauma."

During the Civil War, more than 15% of those fighting enlisted while still under 18, and some were as young as 9. These were 93% more likely than their older comrades to experience later illnesses. Using the percentage of a veteran's company lost to quantify his exposure to trauma, the researchers found that those who lost at least 5% of their company had a 51% increased risk of later development of cardiac, gastrointestinal, or nervous disease.

Field hospital in Virginia.

CREDITS (TOP TO BOTTOM): NASA; BONHAM'S; BETTMANN/CORBIS

DUMBING DOWN

A British education researcher is causing a stir with his report indicating that U.K. children are getting a lot less sharp than they were 30 years ago.

In a study submitted last month to the Economic and Social Research Council, psychologist Michael Shayer of King's College London reports that performance by children of both sexes has plummeted on a test that involves perceptions of weight and volume. Shayer compared the 1976 performance of 2350 11- and 12-year-olds in a representative sample of British schools with that of students from the years 2001–04. "An 11-year-old today is performing at the level an 8- or 9-year-old was performing at 30 years ago," he concludes. In 2004, only 5.7% of boys could equal scores made by the top third in 1976.

The test features questions such as whether the volume of water stays the same when it is poured into different shaped vessels. Psychologist Jim Ridgway of Durham University, U.K., calls it a "fairly robust indicator of cognitive development." Shayer blames the falling scores partly on computer games. Children, especially boys, are playing more in virtual worlds instead of "outdoors, with tools and things," he says.

Durham education researcher Peter Tymms calls the findings "something to be worried about," but says they need confirmation as they are belied by rises in IQ and other test scores.

Hooke Notes for Sale

A rare chronicle of a scientific revolution has been found in a cupboard. The folio of more than 500 pages of meetings minutes and notes, written by the pioneering English physicist Robert Hooke, describe the early years of the U.K.'s Royal Society. The anonymous owner will put it on auction in London on 28 March.

The writings are from 1661 to 1682 when Hooke was the curator of experiments and then secretary of the society. Several scientific breakthroughs are noted, such as the discovery of bacteria in 1676. Dutch microscopist Anton van Leeuwenhoek, Hooke wrote, found "a vast number of small animalls in his Excrements which were most abounding when he was troubled with a Loosenesse and very few or none when he was well."

The notes introduce German mathematician Gottfried Leibniz's idea of a "universal algebra" for encoding logical statements, the founding principle of computer science—along with the society president's observation that the idea could not be "of soe great use as he seemed to suggest." Hooke also takes stabs at his peers, including his rival Isaac Newton. And next to the announcement of a book on navigation by physicist Robert Boyle, Hooke writes, "stoln from me."

Astronomer Martin Rees, current president of the Royal Society, is calling for a "white knight" to buy the folio on the society's behalf. The price is expected to exceed $1.5 million.

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Don't think, decide!
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KOREAN STEM CELL SCANDAL

Schatten: Pitt Panel Finds 'Misbehavior' but Not Misconduct

A University of Pittsburgh (UP) panel has declared stem cell researcher Gerald Schatten innocent of research misconduct in the South Korean stem cell debacle. But his failure to more closely oversee research with his name on it does make him guilty of "research misbehavior," according to a summary report released on 3 February.

In December, after the discovery of misdeeds by South Korean cloning researcher Woo Suk Hwang, UP medical school dean Arthur Levine set up a panel of six senior researchers to investigate the role of Schatten, who was presented as senior author on a paper purporting to show that disease-specific cell lines had been derived using stem cells from cloned human embryos. The paper, published in *Science* in June (17 June 2005, p. 1777), has been withdrawn.

Photo opportunity. Gerald Schatten's (*right*) major contribution to the "Snuppy paper" was to suggest a professional photographer.

The university panel said there is no evidence that Schatten falsified anything or that he was aware of any misconduct. However, it comes down hard on him for "shirk[ing]" his responsibilities when it came to assuring the veracity of the manuscript.

The report relates that Schatten and Hwang first met at a stem cell meeting in Seoul in December 2003 and developed a close relationship, which soon bore fruit for both scientists: It says Schatten's behind-the-scenes "lobbying" of *Science* editors helped assure the publication of a 2004 paper (12 March 2004, p. 1669) on the development of stem cells from a cloned human embryo, a charge *Science* Editor-in-Chief Donald Kennedy denies, saying, "If anything, hearing from Jerry was a distraction."

Schatten had nothing to do with the authorship of the 2004 paper, which was also subsequently found to be fraudulent. But he devoted "a tremendous amount of time and energy" to the 2005 paper, composing numerous drafts and allowing his name to appear as senior author. Despite this, "he did not exercise a sufficiently critical perspective as a scientist," the panel relates.

For example, Hwang told Schatten in January 2005 that some cell lines had been lost through contamination. But Schatten failed to realize from this that there was not enough time to grow and analyze new ones by 15 March when the paper was first submitted. He also failed to ensure that all 25 co-authors had approved the manuscript before submission.

The investigators suggest that Schatten's desire for "reputational enhancement" may have helped land him in his current predicament. For example, in December, he told them that he had written the 2005 paper. But 3 weeks later, he told investigators from Seoul National University (SNU) that he had not. "[T]his appears to be part of a concerted and deliberate effort … to further distance himself from Dr. Hwang and their joint publications," the panel concluded. This it labeled "disingenuous" and "in sharp contrast to the full participation of Dr. Schatten in the media spotlight following publication of the paper."

The panel also takes a swipe at Schatten's role as a co-author on the so-called Snuppy paper, published in *Nature* in August 2005, reporting on the first cloning of a dog. (That achievement was confirmed to be authentic.) "We have no reason to doubt [his] statement to us that his major contribution … was a suggestion that a professional photographer be engaged so that Snuppy would appear with greater visual appeal," says the report. "It is less clear that this contribution fully justifies co-authorship."

Schatten profited financially as well, according to the report, which says, "He was not averse to accepting honoraria totaling $40,000 within a 15-month period from Dr. Hwang—including $10,000 paid in cash" at a press conference on the 2005 paper. These amounts seem "far above normal honoraria for consultation," the panel writes. But it does have a few kind words for Schatten, acknowledging his "expeditious and appropriate actions" upon learning of problems with the paper.

The report recommends no specific disciplinary action, calling on the university to take action "commensurate with … research misbehavior," a term apparently unique to UP. Chris Pascal, director of the Office of Research Integrity of the U.S. Department of Health and Human Services, says universities have a right to add refinements to categories of malfeasance. But Kennedy says, "I think 'research misbehavior' is not a term that anybody in our community understands."

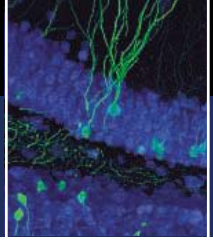
No further details are available from UP, which said no officials would be available for interviews. Schatten continues to maintain the silence he has held ever since he broke off his collaboration with Hwang last November.

Many of Schatten's colleagues in the stem cell world are being restrained in their reactions. "I have nothing to say about this sad situation," says Harvard University researcher George Daley. But Evan Snyder, a stem cell researcher at the Burnham Institute in San Diego, California, believes Schatten was as much a victim as anyone else. "Jerry is kicking himself for having trusted this guy as much as he did," says Snyder. "He knows the buck stopped with him. … I don't think he needs to be slapped on the wrist for being an opportunist." Snyder also says Schatten broke with Hwang immediately after Hwang told him about unethical egg donations.

Back in South Korea, SNU last week suspended Hwang and the six SNU professors listed as co-authors on the 2004 and 2005 papers. They will be barred from teaching and research until an SNU disciplinary committee announces its findings. **–CONSTANCE HOLDEN**

With reporting by Sei Chong.

CREDIT: AHN YOUNG-JOON/AP PHOTO



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2007 U.S. BUDGET

How the Competitiveness Initiative Came About

To many physical scientists, the American Competitiveness Initiative (ACI) announced this month by President George W. Bush may seem like manna from heaven: It would double over 10 years the combined $9.5 billion budgets of the National Science Foundation (NSF), core programs at the National Institute of Standards and Technology (NIST), and the Office of Science at the Department of Energy (DOE), starting with a $910 million boost in 2007 (*Science*, 10 February, p. 762). But it has more earthly political roots. In addition to signaling the Bush Administration's support for basic research in the physical sciences, the initiative provides a window on how this Administration makes science policy.

ACI is a $136 billion package of proposals whose most costly component—an estimated $4.6 billion in 2007 and $86 billion over 10 years—would make permanent a tax credit for companies that increase their research budgets. Its doubling provision would cost $50 billion over a decade. ACI also contains a 1-year infusion of $380 million for the Department of Education to improve math and science in the nation's elementary and secondary schools.

These ideas—and many others—have been blowing around Washington, D.C., for years. Bush has repeatedly sought to make the tax credit permanent, for example, and in 2002, Congress passed a bill that would double NSF's budget over 5 years, although that hasn't happened. The winds picked up in 2005, as a bevy of reports, speeches, and legislation urged a greater federal investment in research and science and math education to sustain U.S. economic might.

In searching for a tipping point that led to the unveiling of the initiative in the president's 31 January State of the Union speech, the media quickly settled on a couple of December meetings at the White House. There, high-tech industry CEOs and scientific leaders discussed a prescription for change laid out in an October report from the National Academies entitled *Rising Above the Gathering Storm* (*Science*, 21 October 2005, p. 423). But the history is more complicated—and more interesting.

Presidential science adviser John Marburger had actually proposed similar funding increases for the same three agencies that are the focus of the initiative—NSF, NIST, and DOE—more than a year ago, on a one-time basis. But White House budget officials were initially cool to the idea. (NSF Director Arden Bement had asked for a 15% boost in that same 2006 budget cycle but was granted only 2.5%.) "There's no question that the physical sciences weren't being funded to take advantage of the opportunities that exist," Marburger told *Science* this week. "The money needs to be very targeted, however."

This time around, Marburger had help from Samuel Bodman, a former Massachusetts Institute of Technology chemical engineering professor and corporate CEO who in January 2005 became Energy Secretary. Bodman lobbied hard for what eventually became a 14% increase for his agency's Office of Science, emphasizing the value of national user facilities such as the synchrotron and spallation sources. Marburger also benefited from the release of the academies' report just as the 2007 budget requests were under scrutiny.

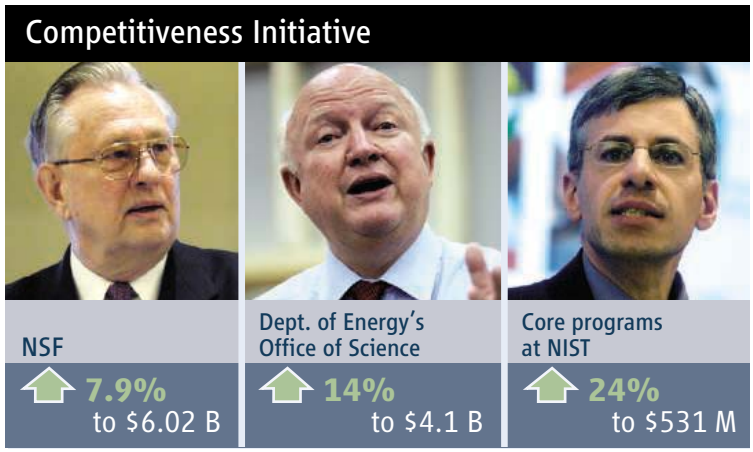
Within days, the president's economic advisers tackled the issue, and by the beginning of December, White House staffers had prepared an initiative for the president. The document also provided talking points for Cabinet secretaries to use at a series of closed-door meetings with CEOs, university presidents, and other stakeholders during a 6 December Innovation Summit organized by the Commerce Department at the behest of representatives Frank Wolf (R–VA), Vernon Ehlers (R–MI), and other science advocates in the House. "Industry was the most recent sector to be brought in," Marburger notes, "although the momentum had been building for some time."

With high-tech executives on board, some in the White House were worried that any competitiveness initiative would be seen as self-interested lobbying for a permanent tax credit. So Chief of Staff Andrew Card sent word to several leaders in the scientific community asking for their views about some type of innovation initiative. Not surprisingly, he received a flood of supportive comments.

After the decision was made in mid-January to have the president propose ACI, the details were held in strict confidence until the day of the speech. Agency heads were told only that they had been selected for a "science initiative," and information was dribbled out by various Administration officials in the 6 days between the president's address and his budget submission. Although her agency is slated to receive only a tiny slice of the ACI pie, Education Secretary Margaret Spellings was given a starring role. Custodian of the Administration's signature No Child Left Behind program and a fellow Texan with strong ties to the president, Spellings led off a hastily arranged press briefing by four Cabinet secretaries the morning after the initiative was announced.

Now that the president has spoken, Congress must decide whether it will give each agency what Bush has requested—and for the designated programs. Despite an overall budget for 2007 that would reduce domestic discretionary spending, Wolf, who chairs the spending panel with jurisdiction over NSF and NIST, flat-out promises that both agencies "will get their number." (NSF is pegged for a 7.9% boost, and NIST's core programs would rise by 24% once projects earmarked by individual members are removed from the budget.) "I don't plan to spend a year talking about it, like we had to do last year," Wolf adds. "We're going to get it done."

–JEFFREY MERVIS



Triumphant trio. NSF Director Arden Bement, DOE Secretary Samuel Bodman, and NIST Chief William Jeffrey applaud the president's new initiative.

SOURCE: 2007 BUDGET; (IMAGES, LEFT AND RIGHT): CHRIS MADDALONI; (CENTER) STEVEN SENNE/AP PHOTO

PALEOBIOLOGY

Revised Numbers Quicken the Pace of Rebound From Mass Extinctions

Paleontologists found it hard to believe that some sort of Darwinian traffic cop was slowing the biosphere's recovery from major extinctions. But that's what the past half-billion years of marine fossils seemed to tell them. Read literally, this history of life said it took 5 million to 10 million years for new species to begin replacing the losses suffered during extinctions. That would be bad news for a modern biosphere battered by a human-induced mass extinction.

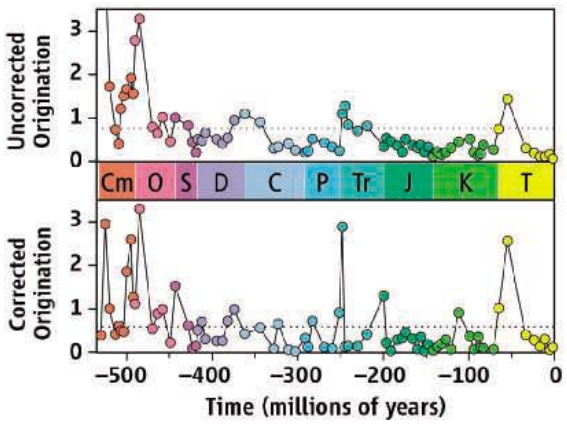
But now researchers have taken a second look at the fossil record after trying to remove some of its imperfections. "The biosphere seems to be more volatile, more responsive to perturbations" than it had seemed, says evolutionary biologist Charles Marshall of Harvard University, an author of the paper in the 21 February issue of the *Proceedings of the National Academy of Sciences*. In this revised history, at least, there's no cop to hold life back.

The new reanalysis was a serendipitous affair. Harvard physics graduate student Peter Lu* learned about the well-known marine fossil record compiled by the late paleontologist Jack Sepkoski while taking courses from Marshall. Lu had "grown up collecting rocks," so the paleontology courses were in the line of recreation. Lu in turn showed the record to his former Harvard roommate Motohiro Yogo, who is now an assistant professor of finance at The Wharton School of the University of Pennsylvania.

Sepkoski's raw data had already been analyzed by geoscientist James Kirchner of the University of California, Berkeley, and paleontologist Anne Weil of Duke University in Durham, North Carolina. Their results suggested "intrinsic limits" on the biosphere that "imply that today's anthropogenic extinctions will diminish biodiversity for millions of years to come," they wrote. Either a post-extinction world is environmentally inimical to life for millions of years, paleontologists speculated, or all that time was needed to rebuild a ruined food web.

Economist Yogo had another idea: Why not analyze the record of life using vector autoregression? That is a technique commonly used to forecast the performance of the stock market or the economy from past behavior. When applied to Sepkoski's raw record of when genera first appeared and last appeared in the record, it suggests that "things move kind of slowly," says Lu; the record displayed the same evolutionary inertia Kirchner and Weil found.

The Harvard group then analyzed a modified version of the record. Paleontologist Michael Foote of the University of Chicago in Illinois had attempted to take account of known biases in the fossil record, such as the varying amount of exposed fossil-bearing rock found in different geologic time intervals. With such revisions, "the speed limit disappears," says Lu. In general, there's no delay between extinction and recovery, although there may be exceptions, such as after the great Permian-Triassic mass extinction.



New lease on life. Jumps in the rate at which new genera appear in the fossil record seem to be delayed and protracted (*top*) until the record is corrected (*bottom*).

The new analysis is being received with a mix of caution and relief. "We all wish we had the real history of life," says Kirchner. "We don't and never will, [so] we try to account for the imperfections." In this latest effort, whether the revised pattern of evolution as analyzed by the Harvard group "is real or artificial is very hard to sort out," Kirchner says. "The error bars can be large."

Paleontologists such as Douglas Erwin of the National Museum of Natural History in Washington, D.C., find the new result "makes a great deal more biological sense than the prolonged delay" of recoveries. However it plays out, "this is the battle line for the next decade in paleontology," says paleontologist Steven Holland of the University of Georgia, Athens. "We're going to see a new wave of analyses that take incompleteness [of the fossil record] into account. Our view of evolutionary patterns is going to change." **–RICHARD A. KERR**

* See profile on www.sciencecareers.org.

CREDIT: P. J. LU *ET AL.*, *PNAS* 103, 8 (2006)

Innovation Craze Hits China

BEIJING—China has unveiled an ambitious 15-year plan for ditching its follow-the-leader approach to R&D in favor of one that prizes innovation.

The plan calls for boosting spending on R&D from 216 billion yuan ($26 billion) in 2004 (1.4% of GDP) to 900 billion yuan ($110 billion) in 2020 (an estimated 2.5% of GDP). The plan identifies 16 state projects, including human space flight and broadband wireless communications, and four priority basic science programs: protein sciences, reproductive biology and development, nanotechnology, and quantum mechanics. Chinese Academy of Sciences biophysicist Zou Chenglu says the blueprint is "generally good. … But it leaves limited room for basic science."

–GONG YIDONG

Grantee Granted Reprieve

The U.S. Bureau of Land Management (BLM) has reinstated funding for a study published in *Science* that determined that logging after wildfires harms a forest's recovery (*Science*, 10 February, p. 761). BLM had suspended the $300,000 grant to Oregon State University (OSU) while it investigated whether the authors had used their paper to lobby against pending federal legislation that would facilitate salvage logging in national forests.

OSU says that a reference to the pending legislation inadvertently left in by *Science* editors was not supposed to have appeared in the online version of the paper.

Representative Greg Walden (R–OR), who has introduced the salvage logging bill, will chair a field hearing in Medford, Oregon, next week on the implications of the paper.

–ERIK STOKSTAD

Taira on Offensive

TOKYO—Kazunari Taira, a University of Tokyo chemist whose research results have been questioned, is fighting back. Last month, a university investigating committee concluded that no one could reproduce the results in several of his published RNA studies (*Science*, 3 February, p. 595). In a 4 February letter, Taira called the committee's report "one-sided [and] exaggerated." He says he was not given an opportunity to respond, and he wants a new investigation. Kimihiko Hirao, the university's engineering school dean, defended the investigation in a statement the same day, pointing out that Taira's group was not able to produce any raw data for the disputed work. Another committee is considering his punishment.

–DENNIS NORMILE

AVIAN INFLUENZA

H5N1 Moves Into Africa, European Union, Deepening Global Crisis

Global anxiety over the H5N1 avian influenza strain ratcheted up several notches last week. European Union authorities called an urgent meeting after the virus was found in dead swans in Italy, Greece, Slovenia, and Austria, the first E.U. countries to be affected, and in Bulgaria. Even more disconcerting, H5N1 has gained a firm foothold on a third continent, Africa, where fighting it will be an uphill battle. By press time, avian influenza had hit poultry farms in at least three states in northern Nigeria. Meanwhile, scientists are puzzled by the apparent lack of evolution of the virus as it hops from continent to continent.

Beset by disease, poverty, and a lack of infrastructure, Africa is ill-equipped to deal with H5N1, says Samuel Jutzi, director of the Animal Production and Health Division at the U.N. Food and Agriculture Organization (FAO). FAO's efforts to prepare veterinary authorities there over the past year have had little effect, he adds, in part because the organization lacked money; large sums were not pledged until last month's donor meeting in Beijing (*Science*, 27 January, p. 456). Last weekend, FAO urged the Nigerian government to tackle the disease more aggressively—for instance, Jutzi says, by deploying police or the military to close poultry markets.

Human health experts are also worried. The majority of Nigeria's chickens live in and around people's homes, so risks of human exposure and disease are high. A World Health Organization (WHO) team arrived in Lagos on Sunday to study the outbreak and provide advice on preventing human infections. WHO's relations with Nigeria have been bumpy after authorities in the northern state of Kano halted polio vaccination in 2003 amid rumors that the vaccine was tainted with sterility drugs, dealing a blow to the international eradication effort (*Science*, 2 July 2004, p. 24). Vaccination resumed a year later after intense diplomatic efforts, and the problems should not interfere with the fight against avian influenza, says WHO's David Heymann. Already, he says, the network of Nigerian polio surveillance officers is helping spread the word about the risk of ill poultry; it can also aid human surveillance.

No poultry have succumbed in the five European countries that reported H5N1 in dead swans this week, and strict biosecurity measures should help keep the virus out of commercial flocks, says Jutzi.

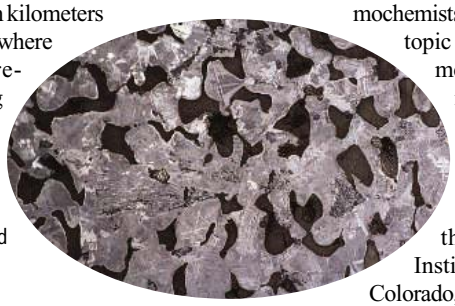
Into Africa. Close contact between people and poultry increases the risk of human infections with H5N1, which has reached Nigeria.

Meanwhile, bird flu researchers are wondering why H5N1, which underwent many genetic changes during its 2-year romp through East Asia, appears almost frozen genetically on its west- and southward march. Sequences from strains isolated in Qinghai Lake in China, and in Mongolia, Turkey, and Nigeria over the past 9 months are almost identical, which is "very, very peculiar," says Michael Perdue, a WHO animal disease expert. One possible explanation is that the virus infects only a few wild bird species, says Perdue, reducing its chances of evolution. WHO hopes to assemble influenza researchers at a meeting in March to discuss H5N1's genetics, he adds. **–MARTIN ENSERINK**

PLANETARY SCIENCE

Hunt for Birthplace of Meteorites Yields New View of Earth's Origins

Citing mounting geochemical data from meteorites and new computer modeling, a group of planetary scientists proposes that the iron meteorites pelting Earth from out in the asteroid belt actually originated in another part of the solar system entirely. Their suspected birthplace—a couple of hundred million kilometers closer to the sun, around where Earth is now—could resolve several nagging problems posed by the asteroid belt.



Earthmaker? Iron like this meteorite may have helped build our planet.

The big conundrum boils down to this: Iron meteorites show every sign of having formed in abundance, but there are few traces in the asteroid belt of bodies in which that could have happened. Iron meteorites are bits of the once-molten cores of planetesimals that got hot enough for their metal to separate and sink to form a molten core. Olivine-rich rock called dunite would have formed a thick encrusting mantle around the cooling, crystallizing cores. But no one can find the dunite, either in other meteorites or in the spectral colors of asteroids.

The dunite dearth and other inconsistencies have prompted decades of debate over how and where planetesimals melted. In the past year, cosmochemists reported a new clue: Isotopic studies showed that iron meteorites formed surprisingly early in the history of the solar system. Early formation, reasoned planetary dynamicist William Bottke of the Southwest Research Institute (SwRI) in Boulder, Colorado, pointed to the inner solar system, well inside the asteroid belt. There, bits of dust could agglomerate into planetesimals the fastest. And the faster planetesimals formed, the more likely they would have been to capture enough short-lived, heat-producing radioactive isotopes to melt them as the meteorite recipe required.

If iron-cored planetesimals formed where the inner planets later came together, why is the iron now raining in from the asteroid belt? To find out how it got there, Bottke and colleagues simulated the fate of close-in planetesimals as the bodies collided with one another and gravitationally flung collisional debris outward. Enough remnants wound up in the asteroid belt to supply the observed flux of iron meteorites to Earth, they found. And asteroid belt collisions would have ground up the weaker stony debris faster, helping deplete the belt's already meager store of dunite. The group reported its conclusions this week in *Nature*.

The idea that planetesimals melted only in the inner solar system and then salted the asteroid belt with a bit of their metallic remains "makes a lot of sense," says asteroid specialist Clark Chapman of SwRI, who knew nothing of Bottke's work until the day before speaking with *Science*. "Nothing flies in the face of what I know about asteroids. If it works out, it would resolve a lot of problems." It would also mean Earth formed from different starting materials—not the chondritic meteorites that dominate collections on Earth, but iron meteorites and their still-elusive stony counterparts. **–RICHARD A. KERR**

CREDITS (TOP TO BOTTOM): AFOLABI SOTUNDE/REUTERS; CHIP CLARK/SMITHSONIAN INSTITUTION, NMNH

GENOMIC DATABASES

NIH Goes After Whole Genome in Search of Disease Genes

The National Institutes of Health (NIH) is ramping up efforts to find genes involved in common diseases. A wave of new projects will take advantage of reduced costs to search for disease genes in people who are already enrolled in existing studies, including the famed Framingham Heart Study. The data will be freely available to other scientists.

Genetic studies on large groups aren't new. But few have searched the entire genomes of participants for common genetic markers called single nucleotide polymorphisms (SNPs). That step is needed to go beyond studying candidate genes to find new genes that slightly raise disease risk.

Using new technologies and the HapMap, a map of human genetic variation completed last year that allows gene hunters to use fewer markers, the cost of such "whole genome association" studies has dropped 30-fold, says Francis Collins, director of the National Human Genome Research Institute. For $3 million, scientists can identify 300,000 markers in 1000 people with a particular disease and 1000 healthy controls, Collins says—enough statistical power to find a gene that raises the risk of that disease by at least 30%. Meanwhile, the recent discovery of genes involved in type 2 diabetes and age-related macular degeneration have spurred the search to identify more common disease genes.

Last week, the 58-year-old Framingham (Massachusetts) Heart Study announced a search for disease genes by scanning for 500,000 SNPs in each of 9000 study participants. Both clinical and genetic data (stripped of identifying information) will be sent to a new Web site at the NIH National Center for Biotechnology Information (NCBI).* Any qualified scientist can obtain the data. "This is taxpayers' money, and the data should be available to many investigators," says Elizabeth Nabel, director of the National Heart, Lung, and Blood Institute (NHLBI), which has allocated $13 million for the project.

Only participants who have given their consent will be part of the genetic database. The new project has avoided the controversy surrounding an earlier proposal to sell access to the study's data, says principal investigator Philip Wolf of Boston University, which runs Framingham (*Science*, 5 January 2001, p. 27).

The same spirit of sharing imbues a new public-private partnership to offer free genotyping services to other disease studies. Drug giant Pfizer has contributed the first $20 million toward a planned $60 million industry-funded effort called the Genetic Association Information Network (GAIN).† Run by the nonprofit Foundation for the NIH, GAIN will begin genotyping this year for up to seven diseases using DNA from existing clinical studies. Investigators who receive funding must allow the linked clinical and genotyping data to be distributed by NCBI right away, but study investigators receive a 9-month head start on submitting manuscripts based on the data.

Added value. The Framingham Heart Study is adding genomic data to its trove of clinical data on local residents.

CREDIT: COURTESY OF FRAMINGHAM HEART STUDY

NIH's parent agency, the Department of Health and Human Services, has proposed in its 2007 budget a $68 million Genes and Environment Initiative. The cross-NIH initiative, with $40 million in new money, would fuel work on several dozen diseases. Whole-genome studies are taking off at individual NIH institutes, too. This week, for example, the National Cancer Institute announced a whole-genome scan for prostate and breast cancer genes.

These efforts should soon resolve whether it's possible to tease out the role of genes in common diseases, says NHLBI's Chris O'Donnell, associate director of the Framingham study: "This will quickly help the world understand the true role of genetic variation."

–JOCELYN KAISER

* www.ncbi.nlm.nih.gov/WGA

† www.fnih.org/GAIN/GAIN_home.shtml

Venture Adventure at NASA

In an effort to return to its 1960s status as high-tech vanguard, NASA is launching a venture capital fund modeled on a similar program set up by the Central Intelligence Agency called In-Q-Tel. That private company was created in 1999 to counter the intelligence community's lag in netting the latest technologies, and Michael Griffin, now NASA's chief, served as In-Q-Tel president from 2002 until 2004. Now Griffin is creating Red Planet Capital, which would combine private and government funds to pinpoint emerging technologies while avoiding bureaucratic barriers. The agency intends to plow more than $10 million into the effort in 2006, with more later. It's looking for private investors in fields such as nanotechnology, robotics, and intelligent systems. **–ANDREW LAWLER**

Stamp of Disapproval

NEW DELHI—A head of a group that campaigns for the free movement of scientists has fallen victim to the U.S.'s tough visa regime. Indian organic chemist Goverdhan Mehta, president of the International Council for Science in Paris, had applied for a visa to visit the University of Florida, Gainesville, for talks and collaborative research. During a routine consular interview last week, Mehta says a U.S. official accused him of "hiding things" and suggested that his research could be applied to chemical weapons work. An embassy spokesperson calls requests "for more information" standard. Mehta says his work is "by no stretch of imagination related to chemical warfare." With a visa still not issued, Mehta has canceled his trip and calls the experience "humiliating." Scientists should participate "without discrimination and on an equitable basis in legitimate scientific activities, including attendance at international meetings," wrote Mehta in *Science* in 2004 (10 September 2004, p. 1531).

–PALLAVA BAGLA

Oui to French Stem Cells

PARIS—Government regulations published last week have paved the way for French scientists to begin deriving their own stem cell lines from human embryos. Until now, researchers could work with imported embryonic lines—about 10 teams are doing so—but could not create their own. "We're quite satisfied," says stem cell researcher Michel Pucéat of the National Center for Scientific Research in Montpellier. The French Biomedicine Agency will supervise the work.

–MARTIN ENSERINK

MEDICINE

Mouse Study Suggests Cancer Drugs Could Help Prematurely Aging Kids

Children with Hutchinson-Gilford progeria syndrome (HGPS) are running out of time. This genetic condition, which is known to affect fewer than 50 children worldwide, causes what looks like premature aging. It produces symptoms such as osteoporosis, hair loss, and atherosclerosis by early childhood and causes death by the teenage years. Since the 2003 identification of the mutant gene responsible for HGPS, scientists, including the mother of a boy with the disease, have rushed to translate that discovery into a treatment. In a paper published online by *Science* this week (www.sciencemag.org/cgi/content/abstract/1124875), a research team led by Loren Fong and Stephen Young of the University of California, Los Angeles (UCLA), reports that a drug originally developed to treat cancer can forestall symptoms in and increase the survival of mice with a similar disease.

"I find this very exciting, and it certainly propels us to consider very seriously the appropriate timing for a human clinical trial for progeria," says Francis Collins, director of the National Human Genome Research Institute, who also studies the disease. "There is a serious time pressure to make a decision and get started." Indeed, Collins's colleagues at the National Institutes of Health (NIH) have been conducting baseline studies on children with HGPS in preparation for such a trial.

HGPS has become a subject of intense research in the past few years, largely through the efforts of Leslie Gordon, a physician whose son was diagnosed with HGPS in 1998. She and her physician husband Scott Berns set up the Progeria Research Foundation in Peabody, Massachusetts, lobbied for funds from Congress, and recruited top scientists (*Science*, 9 May 2003, p. 899). Gordon even joined Collins's lab herself to study the disease after the group had identified the mutated gene responsible.

The typical HGPS-causing mutation occurs in the gene encoding a precursor of lamin A, a protein that provides structure to the membrane of the nucleus. This precursor, prelamin A, is modified by an enzyme, farnesyltransferase; that change directs it to the membrane. There, another enzyme called ZMPSTE24 cleaves the protein to produce mature lamin A. In HGPS, a mutation alters the cleavage site, preventing that process and leading to buildup of the mutant prelamin A in the nuclear membrane. Cells from children with HGPS can have nuclei with distorted shapes, such as bulges and herniations.

Once the HGPS gene mutation was found, scientists quickly theorized that drugs called farnesyltransferase inhibitors (FTIs) offered a treatment. The drugs were intended to combat cancer, as the tumor-promoting ▶

Hopeful sign. Like children with Hutchinson-Gilford progeria syndrome (*far left*), mice with a similar condition develop rib fractures (*left*, arrows). Those treated with a class of cancer drugs do so more rarely (*right*).

CREDITS: COURTESY OF JOHN HURLEY FOR THE PROGERIA RESEARCH FOUNDATION (WWW.PROGERIARESEARCH.ORG); (INSET) L. G. FONG *ET AL.*, *SCIENCE*

EPIDEMIOLOGY

Bush Administration Decides It Can't Afford Children's Study

The White House wants to cancel a massive study of U.S. children's health ordered up 6 years ago by Congress. The 2007 budget for the National Institutes of Health (NIH), submitted to Congress earlier this month by President George W. Bush, contains no money for the effort.

The National Children's Study would have followed 100,000 children from birth to age 21 to explore the environmental causes of diseases such as asthma, autism, and diabetes. Starting with a national random sample of expectant mothers, researchers would measure each child's exposure to everything from chemicals to video games (*Science*, 10 December 2004, p. 1883).

The estimated $2.7 billion study has received $10 million to $12 million a year for planning from the National Institute of Child Health and Human Development and other federal agencies. Seven pilot centers were chosen last fall, with enrollment to begin in spring 2008. Organizers say they need $69 million in 2007 to ramp up toward the goal of 105 sites.

But NIH's budget for 2007 would require planning to end by the start of the next fiscal year in October. "Eventually the nation may see itself in a position" to support the study, says NIH Director Elias Zerhouni. Study director Peter Scheidt says NIH will continue to follow Congress's directive to launch the study, but in a "parallel process" will wind up preparatory work, such as a biomarker database, and look at whether the pilot centers could be used for smaller studies. "We will make the most effective use of what's been done," he says.

Scientists at these seven centers plan to keep working on the study's design and outreach efforts until they hear otherwise. "My hope is that things will change and the study will be mounted," says one principal investigator, demographer Barbara Entwisle of the University of North Carolina, Chapel Hill.

The study's many advocates, from the American Academy of Pediatrics to the March of Dimes, hope to turn things around. "Many of us believe the National Children's Study is of tremendous importance to the future of health in this country, and we will not be accepting the decision to zero it out," says John Porter, a former congressman and chair of Research!America. But some biomedical research lobbyists acknowledge that persuading Congress to give NIH more than the president's flat request is higher on their priority list than lobbying for additional funds for the children's study. **–JOCELYN KAISER**

protein Ras is also modified by farnesyltransferase. FTIs have so far proved disappointing in tackling solid tumors, notes Young. Still, they're relatively nontoxic; one has been tested on children with cancer for more than 2 years.

Last year, four research teams, including the UCLA group and Collins's group, reported that FTI treatment of cells from HGPS children restored nuclei to their normal shape. But would that be enough to slow or stop the disease, progeria researchers wondered?

The study by the UCLA team begins to answer that question. FTI treatment of the team's mutant mice significantly prevented the osteoporosis, slow growth, and loss of grip strength experienced by nontreated mice. The treated group, for example, averaged two rib fractures, whereas untreated mice sustained 14 on average. Moreover, by 20 weeks of age, only 1 of the 13 treated mice had died, compared to 6 of the 14 untreated mice. Fong and Young and their colleagues are conducting larger and longer survival studies, but these early data have impressed others.

"It's a very careful and compelling study," says Susan Michaelis of Johns Hopkins School of Medicine in Baltimore, Maryland, who led one of the teams showing FTIs' effects on a cell culture model of HGPS. "I'm thrilled," adds Gordon. FTIs "look more and more promising for these kids."

Young notes that his group "took a stab in the dark" at an FTI dose and that higher doses might produce even more benefit. He does caution that it's unlikely that FTIs will prevent all the problems found in HGPS. Still, he says, "the families are going to be interested in any improvement, even if it is not a cure."

One caveat to the study is that the mouse strain used by the UCLA team does not have a mutation in the gene for lamin A. It instead has one that eliminates the ZMPSTE24 enzyme. This also results in prelamin A buildup at the nuclear membrane, but these mice may not completely mimic HGPS. For example, they don't develop cardiovascular disease, but progeria children do. "Ninety-five percent die from a heart attack or stroke," notes Collins, who is now testing FTIs on an HGPS mouse model that has some cardiovascular symptoms.

Nevertheless, researchers are debating beginning a trial in the next few months, most likely at NIH's clinical center. "I don't think we're prematurely rushing into this," says Michaelis. "It's reasonable, particularly in light of the extensive baseline studies currently being carried out." Those tests, explains Collins, should help researchers quickly evaluate whether a drug is working.

"It's time," says Young, noting that at a fall progeria conference, he met the parents of a 3-year-old boy with a severe case of HGPS. The child died in January. **–JOHN TRAVIS**

Stop thinking. Too much deliberation can lead to unsatisfying furniture.

CREDIT: STEPHEN CHERNIN/GETTY IMAGES

PSYCHOLOGY

Tough Decision? Don't Sweat It

Buying oven mitts and buying a car demand completely different types of decision-making. Most people would scarcely think about the mitts and agonize over the car. That's exactly the wrong way to go about it, according to a provocative new study.

On page 1005, Ap Dijksterhuis and colleagues at the University of Amsterdam in the Netherlands report a series of experiments with student volunteers and real-life shoppers that suggests that too much contemplation gets in the way of good decision-making—especially when the choice is complicated. Conscious deliberation is best suited for simple decisions such as choosing oven mitts, the researchers argue, whereas complex decisions like picking a car are best handled by the unconscious mind.

"They're elegant experiments with a simple design and eye-popping result," says Timothy Wilson, a psychologist at the University of Virginia in Charlottesville. The research should "stimulate some useful new thinking" among decision researchers, says Daniel Kahneman of Princeton University.

The problem with conscious thought, Dijksterhuis contends, is that you can only think about so many things at the same time. He hypothesized that decisions that require evaluating many factors may be better handled by unconscious thought processes.

To test the idea, Dijksterhuis and colleagues asked volunteers to read brief descriptions of four hypothetical cars and pick the one they'd like to buy after mulling it over for 4 minutes. The researchers made the decision far simpler than it is in real life by limiting the descriptions to just four attributes such as good gas mileage or poor legroom. One of the cars had more plusses than the others, and most participants chose this car. But when the researchers made the decision more complex by listing 12 attributes for each car, people identified the best car only about 25% of the time—no better than chance. The real surprise came when the researchers distracted the participants with anagram puzzles for 4 minutes before asking for their choices. More than half picked the best car. The counterintuitive conclusion, Dijksterhuis says, is that complex decisions are best made without conscious attention to the problem at hand.

To test the idea in a more natural setting, the researchers visited two stores: the international furniture store IKEA and a department store called Bijenkorf. A pilot study with volunteer subjects had suggested that shoppers weigh more attributes when buying furniture than when buying kitchen accessories and other simple products commonly purchased at Bijenkorf. The researchers quizzed shoppers at the two stores about how much time they'd spent thinking about their purchases and then called them a few weeks later to gauge their satisfaction. Bijenkorf shoppers who spent more time consciously deliberating their choices were more pleased with their purchases—evidence that conscious thought is good for simple decisions, Dijksterhuis says. But at IKEA, the reverse was true: Those who reported spending less time deliberating turned out to be the happiest.

Jonathan Schooler, a psychologist at the University of British Columbia in Vancouver, says the study builds on evidence that too much reflection is detrimental in some situations. But "it adds an important insight" by identifying complexity as a key factor in determining which kind of thought process leads to the best decision. Schooler isn't ready, however, to dispense with conscious thought when it comes to complex decisions. "What I think may be really critical is to engage in [conscious] reflection but not make a decision right away," says Schooler.

Dijksterhuis agrees. When an important decision arises, he gathers the relevant facts and gives it his full attention at first. Then, he says, "I sit on things and rely on my gut."

–GREG MILLER

BIOTECHNOLOGY IN CHINA

Doubts Over New Antibiotic Land Co-Authors in Court

BEIJING—A bioengineering triumph at Sichuan University in Chengdu, China, has been dismissed as a "scientific fabrication" by six of the 18 authors who worked on it. But the project chief at Sichuan has hit back: Last week, Qiu Xiao-Qing sued two of his co-authors–cum-critics, charging that they have injured his and his employer's reputations.

After the Sichuan team described a specific antibacterial protein called pheromonicin in the November 2003 issue of *Nature Biotechnology*, Chinese media anointed the discovery as a "major breakthrough in human antibiotics." However, simmering concerns about the high-profile work escalated into a public brawl last month after a critique appeared on a popular Chinese Web site dedicated to exposing academic misconduct.

The fracas centers on an 18 December letter to *Nature Biotechnology* in which the critics—some of whom also have a business dispute with Qiu—allege that the pheromonicin findings were contradicted by data known to Qiu before the article announcing the discovery went into print. They also claim that "some of us" were included as co-authors without their knowledge. In the letter, posted 1 January on the fraud-busting Web site New Threads (www.xys.org), the six say that they were slow to air the charges because they became aware of the paper's defects only after reading a recent Chinese translation.

The authors sent their explosive letter to *Nature Biotechnology* at the urging of Prophet Biopharmaceuticals, says company president Jonathan Shao. Prophet, registered in Wilmington, Delaware, bought rights to develop Qiu's discovery outside China but now feels it was "fooled," says Shao. He adds that he helped translate the critics' letter and that a colleague in China subsequently released the text to New Threads. Since then, three reviews have been launched: at *Nature Biotechnology*; at Sichuan University, which employs Qiu; and at the University of Connecticut Medical Center in Farmington, which employs the second corresponding author, George Wu.

At a press conference last month, Qiu dismissed the charges as part of a commercial disagreement. Four of the letter signers are employed by Chengdu Yanghui Biotechnology, a subsidiary of Sichuan NTC Holdings Limited, which licensed the discovery from Qiu in 2002. But two of them—Zhang Shuhua and Ou Zhenrong—are government researchers at the National Sichuan Antibiotic Industrial Institute, Laboratory of Pharmacology, with no known financial stake in the case. At the request of investors, Qiu provided sample material for an analysis by Zhang and Ou, and he received a copy of their private report after it was completed in March 2003, according to Shao. The report found that the sample had broad antibacterial effects. Critics cite this as evidence that pheromonicin was not "targeted … against specific bacteria," as the subsequent *Nature Biotechnology* paper claimed.

Counterattack. Qui Xiao-Qing dismisses charges by co-authors challenging a report of a new antibiotic published in *Nature Biotechnology* in 2003.

Last week, Qiu sued the two Sichuan Institute scientists in Chengdu's Wuhou District Court, seeking an apology and about $1200 in compensation. Qiu's attorney was quoted in the *Chengdu Economic Daily* as saying the suit singled out the pair because their report on pheromonicin's lack of specificity is being cited by the critics—and it is wrong. In a telephone interview, Qiu told *Science* that the March 2003 report was largely "irrelevant" to his paper, but that he had included its authors "to show respect for their work on the original data," part of which he used in the paper.

The second corresponding author on the *Nature Biotechnology* paper, Wu, a gastroenterologist, says that in retrospect he cannot tell whether the data are sound. The paper's topic—bioengineered antibacterial proteins—is "totally out of my field," he told *Science*. He says he helped translate the report into English and suggested ways to "beef up the experiments with some controls" and "put this together in a presentable way." "Qiu is a friend of mine," he added, but "I have not seen the original data."

At Prophet's request, Zhao Lijun, a biochemist now at the University of North Carolina, Greensboro, says that in 2004 he reexamined the *Nature Biotechnology* paper and the technical analysis of pheromonicin by the National Sichuan Antibiotic Industrial Institute. Zhao says he immediately realized that the claim of specificity in the *Nature Biotechnology* paper could not be right. He adds that Tibet West Pharmaceuticals, a partner of NTC Holdings, tried to replicate the work but failed to do so, concluding instead that the material provided by Qiu was contaminated with streptomycin. Qiu regards this finding as "ridiculous" because the same company had earlier produced a 50-gram quantity of pheromonicin.

Zhao, who says he has no financial stake in this project, charged in a May 2005 letter to *Nature Biotechnology* that Qiu's material was contaminated with streptomycin; the letter is still in review. The journal's editor Andrew Marshall contacted Zhao on 18 January saying he is gathering more information before making a decision. Marshall was traveling and unavailable to comment before *Science* went to press.

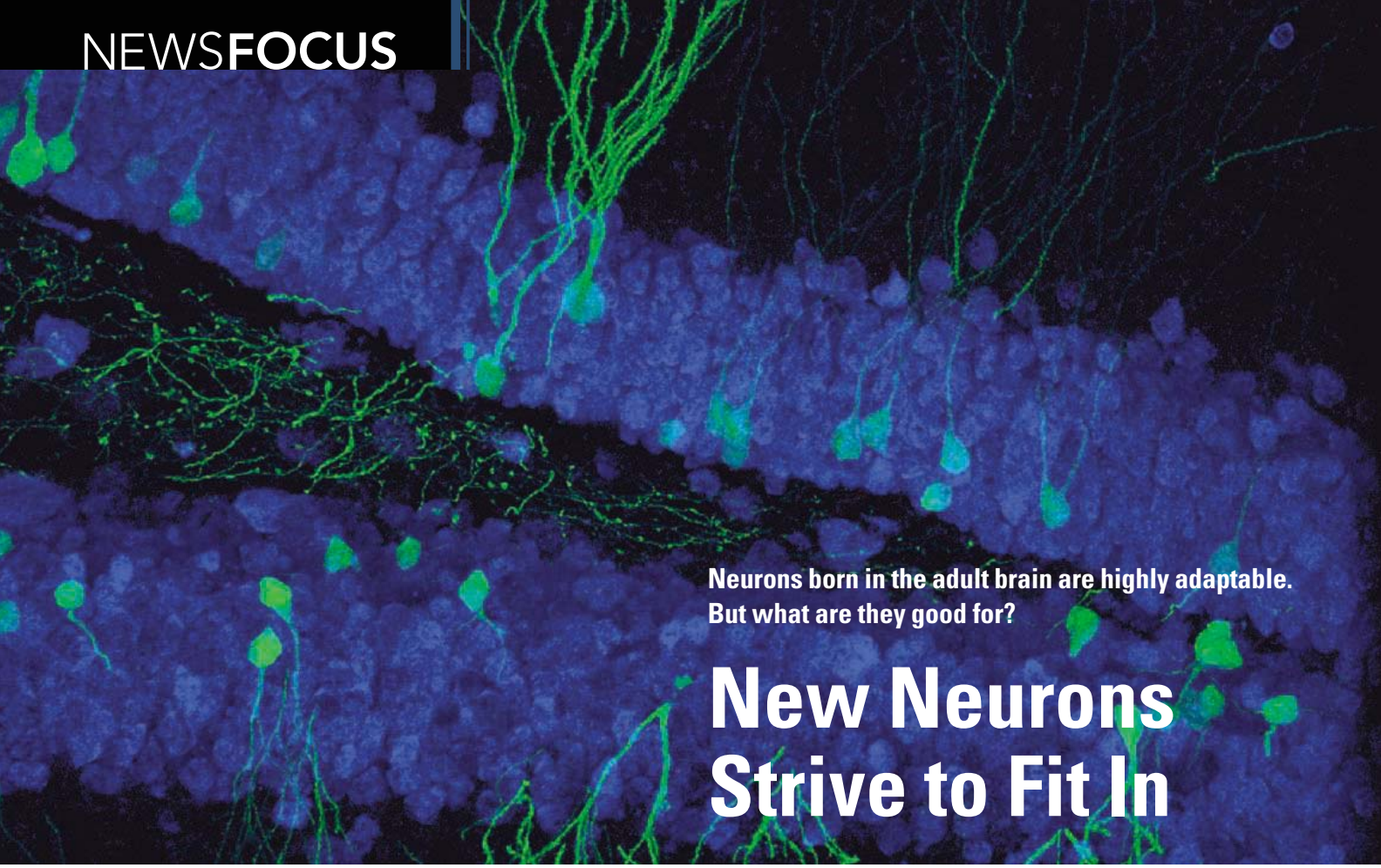
Two other reviews are under way. The University of Connecticut Health Center will decide "within days" whether a preliminary inquiry is warranted, says spokesperson James Walter. Sichuan University announced on 16 January that it had set up an investigation committee composed of university and outside experts, as yet unnamed, but set no timetable.

"Sichuan University regards the safeguarding of academic purity and scientific dignity as being as important as its own life," says university vice-president Li Guangxian. "We will clarify the controversy." Qiu is confident the reviews will vindicate him. "I have nothing to be afraid of," he says, because facts will speak the truth.

–GONG YIDONG AND ELIOT MARSHALL

Gong Yidong writes for *China Features* in Beijing.

CREDIT: YAO YUAN/*CHENGDU ECONOMIC DAILY*



Neurons born in the adult brain are highly adaptable. But what are they good for?

New Neurons Strive to Fit In

IT'S NEVER EASY BEING THE NEW KID IN class. By the time you arrive on the scene, the social network is well-established. All the others know their places and have forged connections with one another. Act shy and you'll never fit in. You've got to be outgoing if you want to survive.

The cruel social dynamics of the schoolyard have a parallel in the daunting situation faced by newborn neurons in the mature brain. Now that the long-held dogma that the brains of adult mammals don't make new neurons has been refuted, neuroscientists are trying to figure out how adult-born neurons integrate into neural circuits that are already up and running. The picture of new neurons that's emerging is one of social gadflies rather than wallflowers.

Compared to older, established neurons, the newbies are hyperexcitable and adaptable. They act in many ways like neurons in the embryonic brain, readily making new synapses with other neurons and changing the strength of these connections. These characteristics seem to mesh well with the popular notion that new neurons play a role in the kinds of brain plasticity that underlie learning and memory. One recent study, for example, suggests that new neurons help adult mice learn novel odors.

"I think there's been huge progress" in understanding how new neurons mature and make working connections with other neurons, says Hongjun Song of Johns Hopkins University in Baltimore, Maryland.

Research on new neurons should ultimately reveal whether aberrations in adult neurogenesis—already described in disorders as diverse as epilepsy, depression, and drug addiction—are a cause or effect of such conditions. Such work also may have implications for future stem cell therapies for brain disorders.

Learning and memory

For decades, neuroscientists thought that the brain was one of the few mammalian organs that doesn't replenish its cells throughout life. This idea fell to pieces in the 1990s when researchers documented newborn neurons in the brains of adult mice and then in human brains. In particular, the hippocampus, a key memory center, proved to be a relative hot spot for neurogenesis in rodents and people. That finding, along with previous work demonstrating neurogenesis in the hippocampi of seed-caching birds and in regions of the songbird brain that are involved in song learning, suggested that new neurons might play a role in learning and memory (*Science*, 3 January 2003, p. 32).

That suggestion has been strengthened by several studies that demonstrated learning impairments in animals whose brains were treated with radiation to kill off dividing cells, or with cancer drugs that do the same. But although the low doses used in these experiments didn't make the animals overtly sick, the treatments may have had effects on the brain other than stopping neurogenesis—making it hard to know the true cause of the learning deficits. To clinch the case, researchers would like to find a way to stop neurogenesis cold without interfering with anything else. New genetic tricks may provide a way to do that.

In October 2005, Fred Gage, a neuroscientist at the Salk Institute for Biological Studies in La Jolla, California, and colleagues reported in *Nature* that Wnt proteins, key regulators of neural stem cells during development, also regulate neurogenesis in the adult hippocampus. When the researchers injected a virus carrying a gene that enhances Wnt activity into the hippocampi of mature mice, neurogenesis increased. A virus carrying another gene that reduces Wnt activity abolished neurogenesis almost completely.

Gage's team is now using this approach to investigate the link between neurogenesis and learning. At a satellite symposium at the annual meeting of the Society for Neuroscience last November in Washington, D.C., Gage's postdoc Sebastian Jessberger presented preliminary results from experiments in which he injected the virus that interferes

CREDIT: VERONICA PIATTI, NICOL S. MORGENSTERN, AND ALEJANDRO F. SCHINDER

Meet the new kids. Adult-born neurons (green) have to carve out a niche among more established cells (blue) in the mouse hippocampus.

with Wnt signaling into the hippocampi of adult rats. These rats performed much worse than untreated rats on a task that tested their recognition of an object they'd seen a week earlier, suggesting that neurogenesis indeed plays a role in long-term memory.

"This is a cleaner approach" to turning off neurogenesis than radiation or chemotherapy drugs, says Jeffrey Macklis, a neuroscientist at Harvard Medical School in Boston. Still, he and others caution that manipulating Wnt could have other effects on the adult brain.

Macklis, along with postdoc Sanjay Magavi and other colleagues, recently reported a different approach to investigating the role of new neurons. Rather than attempting to eliminate neurogenesis, the team studied the behavior of newborn neurons in the olfactory bulb of adult mice by examining the expression of so-called immediate early genes (IEGs), which increases in active neurons. It's very difficult to directly record the electrical activity of olfactory bulb neurons, Macklis says, but IEG expression is widely regarded as a reliable indicator of neural activity.

Olfactory bulb neurons begin responding to odors about 2 weeks after they are born, the team reported in the 16 November *Journal of Neuroscience*. Even though the adult-born neurons are a tiny minority of neurons in the olfactory bulb, they responded to novel odors in larger numbers than did mature neurons. And this difference became even more pronounced with repeated exposures to a once-novel odor: The number of responsive adult-born neurons nearly doubled in a 7-week period, whereas the number of responsive mature neurons declined. The findings suggest that new neurons are essential for learning new odors, Macklis says: "We think it's the first direct evidence of a function of adult-born neurons."

"It's a great paper," says Gerd Kempermann, who studies adult neurogenesis at the Max Delbrück Center for Molecular Medicine in Berlin, Germany. The work shows that new neurons are integrated into the olfactory bulb in a meaningful way and may have a function distinct from that of older neurons, Kempermann says.

Additional evidence supports the learning connection. In 2004, a team led by Josef Bischofberger at Freiburg University in Germany reported in *Nature* that synapses formed by newborn neurons in the adult hippocampus are more malleable than those of more mature neurons. In experiments with slices of hippocampal tissue from adult rats, the team found that new neurons are more excitable than old neurons and can more readily strengthen or weaken their synaptic connections with other neurons—just the type of plasticity neuroscientists think underlies learning and memory.

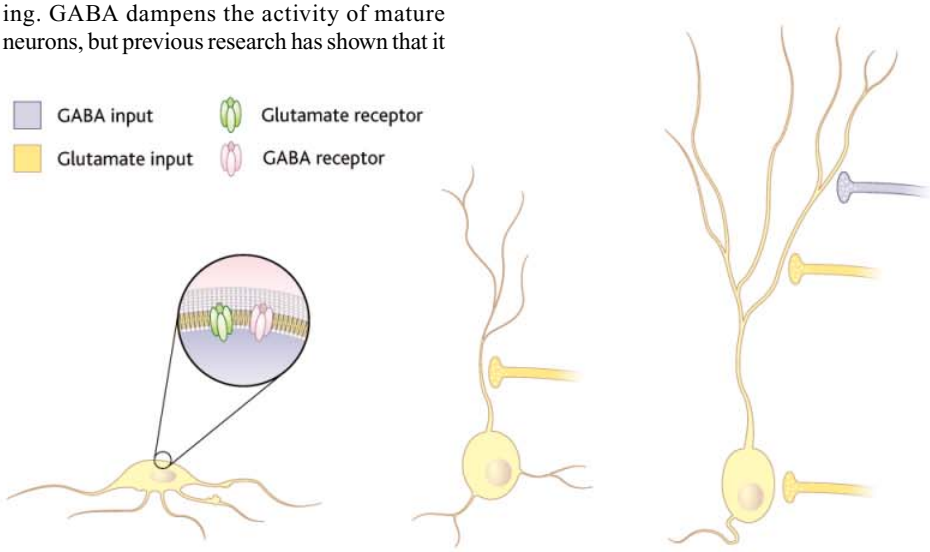
Only the active survive

At a symposium at the neuroscience meeting, Bischofberger and others presented additional details on the physiology of new neurons that suggest activity is a key to survival. Adult-born neurons that don't pick up on the buzz of electrical activity among their neighbors and add something useful to the conversation are less likely to integrate into the existing neural circuitry. And failure to fit in can be lethal to those new neurons, just as it can be in the developing brain, where neural activity helps weed out bad connections.

Song presented work, also published in the 2 February issue of *Nature*, suggesting that the neurotransmitter γ-aminobutyric acid (GABA) plays an important role in this selective weeding. GABA dampens the activity of mature neurons, but previous research has shown that it has the opposite effect on newborn embryonic neurons. Song's team found that GABA also excites new neurons in the hippocampuses of adult mice even before they've formed synapses with other neurons, presumably because receptors on the new cells' surface detect GABA that leaks out from the synaptic connections between older neurons. In essence, the newcomers eavesdrop on the conversations of their elders.

Song's team prevented GABA from exciting newborn neurons by injecting a short piece of RNA that blocked expression of a chloride channel on the neurons' surfaces. This changed the cells' physiology in a way that made GABA inhibitory, as it is in mature neurons. The altered neurons soon developed withered dendrites, the branches that receive inputs from other neurons. If they aren't excited by GABA, the new neurons can't integrate, Song says: "They don't have dendrites, they don't form synapses, and they die."

Another study presented at the symposium suggested that adult-born neurons compete to survive in their new environment—another parallel to brain development. Ayumu Tashiro, a postdoc working with Gage at the Salk Institute, described experiments on NMDA receptors, cell surface proteins sensitive to the neurotransmitter glutamate. Many experiments have implicated NMDA receptors in neuron survival in the developing brain. Tashiro used a combination of genetic manipulations to inactivate NMDA receptors in about 10% of new neurons in the hippocampi of adult mice. He also genetically labeled the cells so he could later determine how well they'd integrated into the hippocampus.

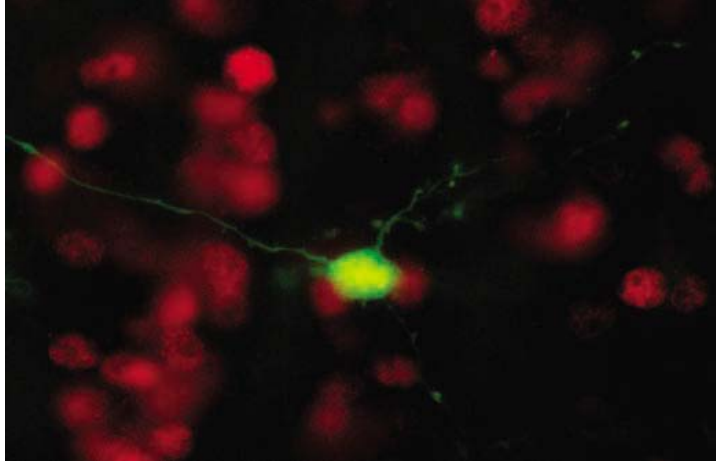


Growing up. Neurons born in the adult mouse hippocampus mature in about 4 weeks and go through a series of stages that mirrors the development of embryonic neurons (*left to right*). Although they initially respond to the neurotransmitters GABA and glutamate, only later do they form synapses with older neurons and produce spikes of electrical activity.

Not very well, it turns out. Many of the altered neurons appeared to be dying 2 to 3 weeks after they were born, Tashiro reported, suggesting that NMDA receptor activation, presumably via synaptic connections from more established neurons, is necessary for survival. Additional work indicated that it's not the overall amount of NMDA receptor activation that counts but the amount relative to other neurons. When Tashiro injected a drug that reduced NMDA receptor activity across the hippocampus, putting the altered new neurons on more equal footing, more of them survived. The bottom line seems to be that new neurons that get good connections survive, whereas those that don't perish—just as they do in the developing brain.

Another study that draws strong parallels to brain development comes from Alejandro

ILLUSTRATION: C. BICKEL/*SCIENCE*, ADAPTED FROM ESPOSITO *ET AL.*, *JOURNAL OF NEUROSCIENCE* (2 NOVEMBER 2005)



Sniff, sniff. Adult-born neurons (green) in the olfactory bulbs of mice respond preferentially to novel odors.

Schinder and colleagues at the Fundación Instituto Leloir in Buenos Aires, Argentina. In the 2 November *Journal of Neuroscience*, the team painted a detailed picture of the maturation of adult-born hippocampal neurons. They monitored the movement and changing shapes of the neurons by labeling them with a fluorescent dye and examined how their electrical activity and responsiveness to different neurotransmitters shifted with time. "The [maturation] sequence is nearly identical" to what happens during embryonic development, Schinder says.

"During early development, there's a critical period where neurons are capable of a greater degree of plasticity," says Linda Overstreet Wadiche, a neuroscientist at Oregon Health & Science University in Portland. Much of the new work is converging on the idea that adult-born neurons recapture this youthful flexibility, Wadiche says: "It's not just that [adult] neurogenesis is adding new cells; it's adding a new type of neuron."

Kempermann theorizes that new neurons optimize the hippocampus to process novel and complex stimuli. Based on his data, Macklis suspects a similar role for new neurons in the olfactory bulb. Both brain regions are ancient structures that help animals deal with novel and complex features of their surroundings, Macklis notes. New neurons may give these parts of the brain additional plasticity that couldn't be accomplished by tweaking existing synapses, as happens throughout the brain. "It makes sense evolutionarily that one would want to … allow whole new circuits to form by the integration of a steady stream of new neurons," Macklis says.

New neurons and disease

A better understanding of the physiology of new neurons in healthy brains should help researchers evaluate the role of adult neurogenesis in the diseased brain as well. An uptick in neurogenesis, perhaps as a compensatory response, has been proposed to accompany several types of brain injury, including stroke and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. There's also evidence that depression reduces neurogenesis and that antidepressant drugs work by promoting it, at least in rodents (*Science*, 8 August 2003, p. 757). Yet little is known about whether newborn neurons in diseased brains successfully integrate into existing circuitry, let alone whether they could be exploited to restore the function of damaged circuits.

In some cases, they may even compound the problem. At the Society for Neuroscience meeting, Wadiche reported that epileptic seizures speed up the maturation of new neurons in adult mice, prompting the cells to form synapses more quickly than usual, and in some cases, to form inappropriate contacts with other neurons. It's a nice demonstration of how pathology can affect new neuron integration, says Song.

Figuring out what new neurons have to do to integrate into the adult brain could have important implications for researchers trying to maximize the brain's limited innate capacity to heal itself or design stem cell therapies for brain injury and disease. Based on what's known so far, however, it may be naïve to expect that just plopping some neural stem cells down will do the trick, says Macklis: "If we're going to repair neural circuits, we're going to have to very carefully activate new neurons so that they're incorporating into the existing circuits."

–GREG MILLER

SCIENTIFIC COMMUNITY

Scientists' Suicides Prompt Soul-Searching in China

A spate of deaths has raised questions about whether China's scientific community is piling too much stress on young researchers

BEIJING—Mao Guangjun seemed destined for scientific stardom. In September 2001, the 32-year-old theoretical physicist, just home after a postdoc stint in Japan, signed a 3-year contract as a full professor with the Institute of High Energy Physics (IHEP) of the Chinese Academy of Sciences (CAS) in Beijing. However, his personal and professional life soon soured, and in 2004, IHEP declined to renew his contract. He landed a position at another university but would never report for duty: On 14 September 2005, Mao, 36, jumped to his death from the fourth floor of his apartment complex.

Stress relief. Some young scientists need help to cope with increased pressures, says Li Daguang.

Although academic stresses weighed on Mao, family members and colleagues told *Science*, no one can say for certain whether those pressures caused him to take his own life; he did not leave a suicide note. But Mao's death and those of a handful of other young researchers in recent months have lifted the lid on simmering discontent among young scientists in China. Their concern is that some institutions, in pressing to gain on the West, are making life intolerable for vulnerable researchers. That's a hot topic these days on Web sites frequented by Chinese academics, including www.sohu.com, a Beijing-based information clearinghouse, www.xys.org, a U.S.-based site aiming to expose fraudulent academic behavior in China, and www.chinahexie.org, an information site run out of China's Guangdong Province. In the words of one anonymous researcher on China Hexie, "Mao Guangjun's death reflects the flaws of the current management system for Chinese intellectuals."

The wave of introspection has prompted some academics to question China's newfound obsession with a sacred cow of Western science, publish-or-perish. And the suicides have

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focused attention on a clutch of programs launched in the last decade to entice young Chinese scientists working abroad to come home. The most competitive, the One Hundred Talent Project, provides young stars with generous lab-start-up funds, housing allowances, and salaries higher than those of some senior scientists. Although the program has no formal requirements for awardees to demonstrate their value, there is plenty of informal pressure on them to prove their mettle.

The stresses at elite institutes like IHEP are now nearly on a par with those felt in the West, scientists say. It is no longer the age of the "Big Rice Bowl," says Zhang Zongye, a CAS academician at IHEP. "Competition has become increasingly intense and unavoidable," she says.

Disqualification

Returnees like Mao tend to get preferential treatment, whether or not they receive One-Hundred-Talent status. After earning a Ph.D. from the China Institute of Atomic Energy in 1995, Mao spent 2 years in Germany as a Humboldt Research Fellow, then 18 months in Japan on a fellowship. "Mao was a good young scientist, very focused and did not have any big flaws," says Zhao Enguang, Mao's postdoctoral supervisor at the CAS Institute of Theoretical Physics in Beijing.

When Mao returned to China at the end of 2000, his Ph.D. adviser, Zhuo Yizhong, recommended him to IHEP. At the time, the institute had few scientists in their early 30s, says Zhang. A committee of IHEP and outside experts endorsed hiring him as a full professor. In hindsight, Zhang says, it would have been better to have started him off as an associate professor, with fewer expectations. People returning from abroad usually need time to settle down and readjust to China's work environment, adds Wu Ke, a mathematician at Capital Normal University in Beijing.

Several factors conspired to make Mao's transition difficult. For starters, once at IHEP he had to move from nuclear physics into an unfamiliar but trendy field, nuclear astrophysics. After such a change, says Zhuo, "it would have been difficult for him to produce any significant results within 3 to 5 years." And Mao was having problems at home. Both Zhuo and Mao's mother say his marriage failed, he slept poorly, and reading gave him headaches. Nevertheless, Zhuo says, Mao recovered and was able to concentrate again on his work.

In October 2004, a 14-member expert committee met to review Mao's work. His output in 3 years consisted of four papers—including three in Chinese journals—and a monograph of his pre-IHEP research. According to a statement issued by IHEP after Mao's death, 11 panel members voted to "disqualify" him from his position. The statement notes that the panel considered Mao's academic achievements, research program, future plans, and academic ability.

Zhuo contends that IHEP's evaluation system, which puts a premium on short-term achievement, is "heavily flawed." Zhao agrees and points to what he views as a growing problem in China. "It is not appropriate to judge a scientist's work merely by the number of papers he has published, nor is it convincing to attach too much importance to overseas publications," he argues. "It is more important to look at the quality of papers instead of quantity." Zhang, who was on the committee, responds that the panel did not base its judgment solely on Mao's publications. "We also looked at his ideas guiding his current work and found them not so promising," she says. The panel's decision could not be appealed.

With help from Wu Ke, in July 2005 Mao landed a job as an instructor at Beihang University, also known as Beijing Aeronautics University. But before the start of the autumn term, Mao ended his life.

Frustration

Another cautionary tale is that of Wu Jianyi, a 39-year-old specialist in plant breeding at the Hunan Academy of Agricultural Sciences. Shortly after joining the academy in 1999, Wu's department was spun off as a company called Xiangyuan Guaguo (Melon and Fruit) Seedling Co. Ltd. His wife, Feng Zhili, recalls that Wu complained repeatedly that his skills lay in research, not marketing. Nevertheless, Wu's research budget was pegged to earnings derived from seed sales.

At a company meeting in late March 2004, Wu was berated for not bringing in enough revenue to cover his own salary, says Feng. A week later, he leaped to his death from the top of their five-story apartment building. In the 2 months before Wu's suicide, two other academy researchers, aged 39 and 41, also killed themselves. Academy officials deny that the deaths were work-related.

Others see a disturbing pattern. The tragedies at the Agricultural Academy and IHEP suggest that some young scholars are too brittle to cope with today's academic pressure cooker, says Li Daguang, a professor of science communication at CAS's graduate school in Beijing. Young stars who have worked abroad claim they are especially vulnerable. In comments posted to www.lqqm.org, one young scientist described the difficulties he encountered after returning to China when he was 29 years old to take up a professorship, sponsored by the One Hundred Talent Project, at the Institute of Modern Physics in Beijing. Soon after his return, he wrote, he often thought of jumping from his office window. His solution to his despair was to take a job abroad. "The research environment in China is still not as good as in Western countries," he wrote. "People coming back from abroad have to deal with *guanxi* [personal relations] if they want to get research funding and other things needed for their experiments."

The bottom line is that more care must be taken to safeguard the physical and mental well-being of young scientists, says Li. Zhang agrees that counseling should be available for young scientists who struggle to maintain their mental equilibrium in intense work environments. And younger scientists should shoulder lighter burdens, argues Zhao. Young people are more likely to be creative, but they often lack management experience, he says. Asking young scientists to run a project before they are mature is a recipe for disaster, Zhao says. For Mao, that lesson has come too late.

–DING YIMIN

Ding Yimin writes for *China Features* in Beijing.

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Pressure cooker? An emphasis on publication records and other measures commonly used in Western countries have added to the demands on young scientists in Chinese labs.

RETROVIRUS MEETING

Novel Attacks on HIV Move Closer to Reality

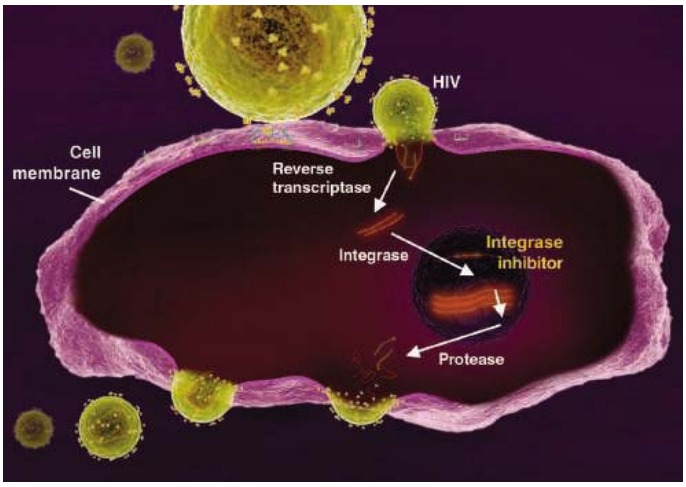
At a recent conference, AIDS researchers reported new insights into the epidemic's origins and progress on treatment and prevention

DENVER, COLORADO—Neither the hunt for an AIDS vaccine nor the search for a cure has made much progress lately, but at the 13th Conference on Retroviruses and Opportunistic Infections held here last week, researchers reported several advances that may lead to novel ways to treat and prevent HIV infection, as well as to a clearer understanding of the epidemic's origins.

Perhaps most encouraging, said one of the meeting organizers, Constance Benson of the University of California, San Diego, a powerful new drug that cripples HIV's ability to weave itself into human chromosomes has made an important leap forward in human trials. The drug, made by Merck and dubbed MK-0518, inhibits the integrase enzyme, which the virus requires to copy itself. The antiretroviral drugs now on the market target different parts of HIV's life cycle.

Beatriz Grinsztejn of the Oswaldo Cruz Foundation in Rio de Janeiro, Brazil, described a multisite, placebo-controlled clinical trial of MK-0518 that involved 167 people infected with multidrug-resistant HIV. MK-0518 lowered the level of HIV to below 400 copies per milliliter—a 99% drop—in 80% of the treated participants. "That's a tough population, and to get 80% [of patients] below 400 copies is about as good as it gets," said Michael Saag, who heads the AIDS research center at the University of Alabama, Birmingham (UAB).

A smaller, shorter study reported last fall revealed that the drug was also safe and potent in HIV-infected people who had never taken an antiretroviral. If the drug works in larger trials and serious toxicities do not surface, said Benson, "it will change the paradigm of treatment." Merck is launching two studies in several countries to assess MK-0518's safety and efficacy in large groups of people with drug-resistant infections and hopes to seek U.S. approval next year.



New target. An integrase inhibitor may soon join the arsenal of anti-HIV drugs that attack reverse transcriptase, protease, and viral entry.

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Prophylactic progress

A monkey experiment of two anti-HIV drugs already on the market, tenofovir and FTC, shed new light on a promising prevention strategy. Researchers are staging six trials around the world in HIV-*un*infected but high-risk people to see whether tenofovir alone can work as so-called pre-exposure prophylaxis (PrEP), thwarting the virus if a person is exposed. Early studies in monkeys with tenofovir PrEP demonstrated its potential, although last year, researchers from the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, reported that the protection didn't seem robust. Tenofovir PrEP solidly thwarted an intentional attempt to infect the animals once, but by the 15th "challenge" with the AIDS virus, no animal remained protected.

In a new study of the same design, CDC's Walid Heneine and co-workers added FTC to tenofovir (a combination drug sold commercially as Truvada) and found that all six monkeys remained protected after 14 challenges. "I think it's fantastic," said Myron Cohen of the University of North Carolina, Chapel Hill. "It's an extremely compelling piece of work that raises the ante of what we should test."

HIV's origins

Two groups studying wild chimpanzees in Cameroon reported progress in deciphering HIV's origins. These teams previously had discovered persuasive evidence that chimps harbor a simian immunodeficiency virus called SIVcpz that became HIV-1—the predominant cause of AIDS in humans—but they had found precious few infected animals with which to make the case. By analyzing 1300 fecal samples from wild apes, the groups found SIVcpz in several geographic areas and then genetically characterized dozens of new isolates. "They're closing in on some very hot stuff," said James Hoxie, who studies HIV and SIV at the University of Pennsylvania. "It's compelling genetic evidence."

Dirty work. To find new isolates of the virus that evolved into HIV-1, researchers in Cameroon collected more than 1000 samples of ape feces.

The new work found more than 30 strains of SIVcpz, tripling the number previously discovered. The researchers took advantage of the fact that chimps cannot swim, which means that rivers naturally separate different communities and block the spread of viruses. For the first time, they found chimp communities in which SIVcpz infection was widespread—in one, up to 35% of the individual animals analyzed had the virus in their feces. "Our eyeballs popped out of our heads," said Brandon Keele, who works with Beatrice Hahn at UAB. In a separate presentation, Fran Van Heuverswyn, part of a team headed by Martine Peeters of the Institut de Recherche pour le Dévelopement in Montpellier, France, described how two of the isolates more closely matched HIV-1 causing the human epidemic than any found in the past.

Building on the new data, Paul Sharp, who studies molecular evolution of pathogens at Nottingham University in the U.K., went a step further back in time to explain the origin of SIVcpz. Researchers have discovered different SIVs in more than 30 species of monkeys. Sharp's new analysis suggests that the SIVcpz closest to HIV-1 is a combination of SIVs isolated from red cap mangabeys and monkeys from the *Cercopithecus* genus.

Filling in the final piece of the origin puzzle, Sharp said the virus must have reached a major city to start the AIDS epidemic. He posited that a person became infected in rural Cameroon and then traveled by river to Kinshasa, Democratic Republic of Congo. Kinshasa has the greatest genetic diversity of HIV-1, suggesting that the virus has been there longer than anywhere else. It also was home to the first known HIV-infected person, a Bantu man who had his blood sampled in 1959 for a malaria study.

–JON COHEN

Basic training. A technician in Phnom Penh's new avian diagnostics lab prepares to test samples of duck blood for bird flu antibodies.

AVIAN INFLUENZA

Combating the Bird Flu Menace, Down on the Farm

A race is on to beef up avian influenza surveillance and train scientists in some of the poorest corners of Southeast Asia

VIENTIANE AND PHNOM PENH—According to Buddhist tradition, a pinch of salt repels evil spirits. For Maha Ouan, lime does the trick. On a farm 15 kilometers northeast of Vientiane, the capital of Laos, Ouan sprinkles the disinfectant on dirt tracks and around chicken enclosures to repel an evil not found in sacred Buddhist writings: the H5N1 avian influenza virus. In January 2004, the dreaded strain swept into this impoverished country, wiping out 45,000 chickens before a mass cull smothered the outbreak. Ouan got lucky: The avian reaper bypassed his 18,000 egg layers. He realizes that was a fluke, though, and isn't taking any chances. "Who knows whether bird flu will come back," he says.

Odds are it will. Few Laotian farmers share Ouan's zeal for hygiene and for keeping livestock penned. Chickens, ducks, and the occasional scruffy hog outnumber people along the road back into town, lined with rippling flags, always in pairs—Laos's full moon over a blue Mekong River and the Communist rulers' Soviet-esque hammer and sickle against a red background. Free-ranging animals "are a very serious concern" because they can fan the flames of an outbreak, says David Castellan, a poultry expert with the California Department of Food and Agriculture in Sacramento, who spent 6 weeks in Laos late last year.

So far, Laos and neighboring Cambodia appear to have been spared the worst. Laos has not detected bird flu since 2004, and not one person has tested positive for H5N1. Cambodia has had only sporadic flare-ups among birds and just four human cases. Yet, the regional powers that sandwich them—Thailand and Vietnam—have grappled with frequent outbreaks and a large share of the global total of 91 human deaths attributed to H5N1 since 2003. This strain is more likely to rear up on large commercial poultry farms, which are common in Thailand and Vietnam, experts say. But the quiet in Cambodia and Laos does not mean that health officials here can afford to be complacent. "Something could happen at any time," notes Somphanh Chanphengxay of Laos's Ministry of Agriculture and Forestry.

Thanks to a $1.9 billion windfall, pledged by the United States, the European Union, the World Bank, and other donors in Beijing last month (*Science*, 27 January, p. 456), Laos, Cambodia, and other Southeast Asian nations are redoubling efforts to strengthen surveillance, both by training health workers on what to look out for and by equipping new labs. The cash infusion will help these countries transform their scientific corps, greatly bolstering their capacities to anticipate and track future disease threats. In some areas, they are starting from scratch: Laos, for example, plans to set up its first-ever faculty of veterinary science at National University in Vientiane.

The scientific buildup here is coming not a moment too soon. "Southeast Asia is the first line of defense" against a potential pandemic, should the H5N1 strain acquire the ability to pass easily among people, says Finn Reske-Nielsen, U.N. resident coordinator for Laos. "There has to be a minimum capacity to say, 'Something funny is going on here. This has to be reported.' " He hopes to see adequate surveillance put in place over the next several months. "It's a huge, huge challenge."

Paragon of poultry. Maha Ouan is one of the few farmers in Laos taking measures to stem the spread of bird flu, such as keeping chickens caged (*inset*).

Extreme makeovers

In a modern lab in the heart of Phnom Penh, two technicians squeeze drops of duck blood from syringes into small plastic vials. The women, staff members of Cambodia's National Animal Health and Production Investigation Centre (NAHPIC), are going to spin the blood down and probe the serum for avian influenza antibodies. Outside, the squeals of children at play can be heard from a primary school next door. "We have to be very careful here," says NAHPIC's director, Sorn San.

A year ago, the Cambodian government was incapable of routine surveillance. Before H5N1 descended, the government had no facility to detect

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any strain of bird flu and had few staff members qualified to work with infected specimens. "A lot has happened since then," San says.

Over the past several months, Cambodian authorities have outfitted an avian influenza diagnostic lab at NAHPIC, using funds raised over the past 2 years by the U.N.'s Food and Agriculture Organization (FAO). The center now has 31 staff, most of whom have B.Sc. degrees from the Royal University of Agriculture. "They're young and need more expertise," says San, one of the few intellectuals who survived the brutal Khmer Rouge years before earning a veterinary degree in Cuba in the 1980s.

Nowadays, hundreds of Cambodians each year stream overseas to earn advanced degrees. "The problem is that few come back," says a U.N. official. Laos, too, suffers an acute shortfall of young scientists. Most Laotian researchers are in their late 30s or older, having earned degrees in the East Bloc, says Chanphengxay, who trained in Hungary. When the Soviet Union collapsed, opportunities for training dried up. In Laos, says Reske-Nielsen, "basically there are five people in the Ministry of Health who are qualified" to deal with bird flu.

FAO is helping close the expertise gap, in part by sending young scientists from Cambodia and Laos abroad for training. And it has sent in a one-man cavalry: Huaguang Lu, an avian virologist at Pennsylvania State University, University Park, who in 2002 developed a cheap dot-ELISA (enzyme-linked immunosorbent assay) test for rapidly identifying the fast-mutating H5 and H7 subtypes of bird flu. Lu has assisted San in setting up NAHPIC's lab, teaching staff how to handle avian influenza samples, isolate viruses in chicken embryos, run dot-ELISA to detect the H subtype, and use agar gel immunodiffusion to check for antibodies. A planned expansion later this year, paid for by funds raised in Beijing, will enable researchers to carry out the polymerase chain reaction (PCR) for rapid diagnosis of H5N1 and add a walk-in cooler and an incubator room. The NAHPIC lab "will be one of the most advanced avian diagnostic labs in the region," says Lu.

Epidemiologist Wantanee Kalpravidh, FAO's avian influenza coordinator for Southeast Asia, is impressed with how far Cambodia has come in such a short time. "Lu is very, very energetic," she says. And he's persuasive, too: "When he asks for something," such as convincing officials to hire more technicians or provide more lab space, "people cannot resist," she says.

Lu is helping establish a similar facility in Vientiane. When chickens began dying in droves in early 2004, Laos lacked the capacity to confirm H5N1 as the culprit; that was done in Thailand. "They never had a virology lab in their history," Lu says.

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The need for handling nasty pathogens was great, though, even before H5N1. Laos has struggled to stamp out foot-and-mouth disease, which prevents it from legally exporting beef and buffalo meat. Laos is also grappling with swine fever and hemolytic septicemia. To boost surveillance of these perennial foes, the Ministry of Agriculture and Forestry a few years ago began building a lab at the National Animal Health Centre (NAHC) in Vientiane. But Laos's economy tanked just before avian influenza struck, halting construction.

On NAHC's grounds overlooking the Mekong, the unfinished three-story lab center, a concrete frame with brick walls but no windows, sits behind a dilapidated corrugated steel fence. Center staff, some wearing Adidas tracksuits, toil in cramped quarters next door. The bird-flu lab was carved out of two tiny rooms in NAHC's 75-year-old main complex. Funds channeled through FAO have enabled NAHC to install a biosafety hood for handling virus-laden blood, and Lu has trained staff to run the same virus-isolation, dot-ELISA, and antibody tests used in Phnom Penh. The Laos government is seeking about $800,000 from foreign donors to complete construction of the new building.

Sign of the times. An economic downturn has prevented Laos from finishing construction of this animal-health laboratory.

Like its counterpart in Cambodia, NAHC's lab is strictly for animal samples. "We still don't have the capacity to make diagnoses in humans," says Sithat Insisiengmay, a microbiologist at Laos's Ministry of Health. He hopes to dispatch staff to the United States for training and at the same time upgrade biosafety in a health ministry lab to allow it to work with tainted human blood. Samples from five suspected cases since 2004 were sent to Tokyo for testing; none were positive for H5N1. Insisiengmay hopes Laos will be able to do PCR on denatured human blood by the end of 2006.

Casting a wider net

The most urgent need is heightened vigilance. "We worry a lot about undetected outbreaks in small backyards," says Chanphengxay. This blind spot isn't restricted to Cambodia and Laos. Myanmar hasn't reported a single bird flu case in poultry or people. Given its brittle relations with the country, FAO cannot judge whether Myanmar's surveillance is up to snuff.

A lucky find in Cambodia illustrates how easy it is to miss an outbreak. Last August, a team from NAHPIC and the Pasteur Institute in Phnom Penh, on a routine surveillance mission, drew blood from a couple of dozen young ducks from a domestic flock in Prey Vêng province, about 80 kilometers east of the capital. Some samples had antibodies to H5, prompting a closer look. When researchers followed up last October, they discovered that roughly 600 of 800 ducks in the flock had died the previous July. "I was shocked," says the Pasteur's Sirenda Vong. "When we were there in August, nobody had reported this to us." They expanded the study, drawing blood from 41 flocks and interviewing farmers. More than half the flocks had been hit with mass die-offs, and antibody tests nailed H5N1. "The virus was all over the place," Vong says.

Detecting future H5N1 outbreaks in ducks and tracking the virus's origins are top priorities, says Vong, a 41-year-old medical epidemiologist who returned to Phnom Penh in late 2004 after a 4-year stint at the U.S. Centers for Disease Control and Prevention in Atlanta, Georgia. In December, Vong's Pasteur team, working with NAHPIC, deployed about 100 sentinel ducks at a lake near Kampong Cham, 100 kilometers northeast of Phnom Penh; they plan to swab cloacae and tracheas and draw blood from the tagged ducks twice a month. And Pasteur plans to work with the Wildlife Conservation Society to look for H5N1 in excrement of migratory birds that pass through Cambodia on their way to northern breeding grounds.

But although Pasteur has put a watch on Prey Vêng and Kampong Cham, it can't keep tabs on all of rural Cambodia. "If people aren't reporting bird deaths, we're not going to detect anything," Vong says. It's unknown, he says, how often outbreaks in ducks flare up and die out on their own.

Hand in hand with better monitoring is the need to raise public awareness of the H5N1 threat. "We need to reach families," says Insisiengmay, who notes that in Laos alone, 85% of poultry is

kept in backyards. To disseminate bird-flu warnings more widely, the government is now translating data sheets in Lao into languages of ethnic minorities, including the Hmong and Khmu. Laos's diverse ethnic groups "pose a huge challenge for risk communication," says Castellan.

Last December, Castellan worked with FAO to train health workers in the Laotian hinterlands, including Champasack, a high-risk province bordering Thailand and Cambodia that was hit hard in 2004. "This is where the rubber hits the road," he says. Their messages were simple: Raise chickens, ducks, geese, and other poultry separately, sell live poultry separately from processed poultry products, and practice good personal hygiene.

However, "it's very difficult to change the minds of farmers," says San. For example, rural Cambodians have no fear of eating chickens that die of Newcastle disease. In unvaccinated flocks, this virus has a mortality rate exceeding 50%, rivaling that of H5N1. "It's hard to distinguish between Newcastle and avian influenza"—even for a veterinarian, says San. The best strategy, he says, is to insist that chickens that succumb to disease are buried, not eaten. "We're trying to train the village health workers to put a stop to that," San says.

Transforming rural lifestyles "won't happen overnight," Castellan says. But on the front lines of Southeast Asia, the battle to stave off a pandemic flu strain is likely to be won or lost not in the cities, but down on the family farm.

–RICHARD STONE

INTELLECTUAL PROPERTY

What Good Is a Patent? Supreme Court May Suggest an Answer

Two cases raise fundamental questions about the scope of a patent and the right balance between protecting innovation and hindering commerce

Next month, the Supreme Court will hear two cases that could punch holes in a strong patent regime credited with fostering the remarkable growth of the U.S. biotechnology industry. Experts predict that the high court may rein in a specialized lower court that has shaped U.S. patent policies for the past 2 decades. At a minimum, the court's involvement reflects a world increasingly dependent on intellectual property.

The two cases pose key questions about what can be patented and the force a granted patent should have in the marketplace. In *Laboratory Corp.* v. *Metabolite*, a case involving two makers of diagnostic blood tests, the high court will probe the limits of patenting basic scientific principles. In *eBay* v. *MercExchange*, the court could rule on how much a patent holder can interfere with the activities of a company or organization infringing on its patent. (A third case up for review this spring would give the court a chance to decide how obvious a proposed invention must be to be denied a patent.) Together, the cases could have "major, major impacts" on existing patents and future applications in a range of disciplines, says former U.S. commissioner of patents Nicholas Godici of Birch, Stewart, Kolasch & Birch LLP, based in Falls Church, Virginia.

The cases come amid calls for reforming a system bogged down by questionable patents and expensive lawsuits. Although a 2004 report by the National Research Council of the National Academies concluded that the system "does not require fundamental changes," it warned that further deterioration of patent quality could "impede research progress" and discourage innovators from "invent[ing] and disseminat[ing] technology." Last year, Congress took up the issue, but disagreements over patent quality and the appropriate use of injunctions against violators derailed proposed legislation (*Science*, 17 June 2005, p. 1725).

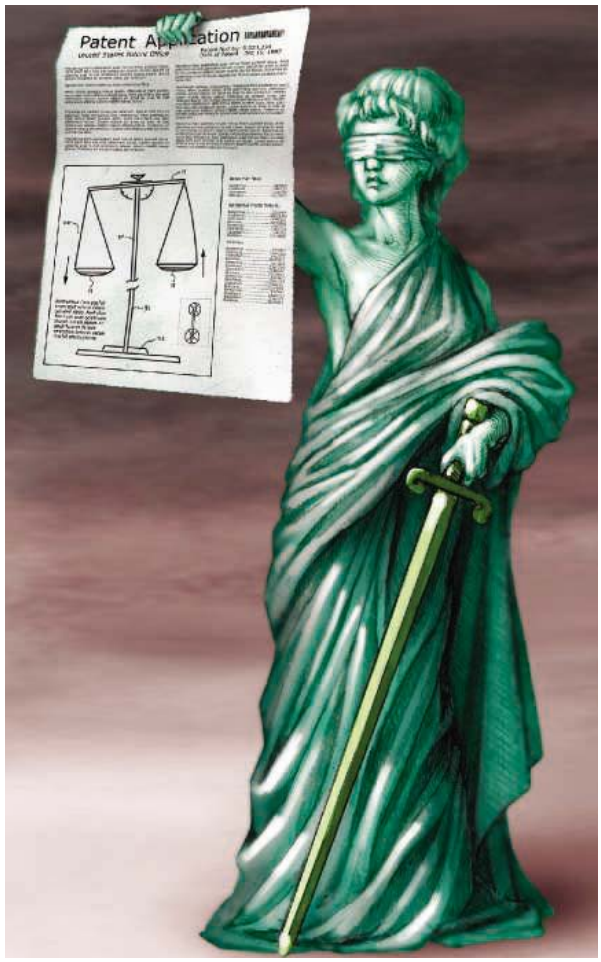


ILLUSTRATION: CAMERON SLAYDEN/*SCIENCE*

The framers of the U.S. Constitution included patents as a way "to promote the progress of science." For nearly 2 centuries, the United States has had some of the strongest patent rights of all nations. But since the founding of the specialized Federal Circuit appellate court in 1982, the system has struggled to find the best way to protect discoveries in biotechnology, information technology, and other emerging fields.

Because the Supreme Court rarely intrudes on the appellate court's turf on major issues, patent lawyers say its decision to accept the two cases suggests that the justices might want to step in and review how far the lower court has gone. But some patent lawyers turn queasy at the thought of having nine "outsiders" take on the system. "They think the [Federal Circuit] needs to be tightened down," says attorney Vern Norviel of Wilson, Sonsini, Goodrich & Rosati in Palo Alto, California. Under a worst-case scenario, says Kevin Noonan of McDonnell, Boehnen, Hulbert & Berghoff LLP in Chicago, Illinois, the court's upcoming rulings could imperil "thousands of [granted] patents" and "harm innovation."

Can you patent nature?

The first case centers on defining what is a natural phenomenon and, therefore, not patentable. Metabolite has rights to a patent for measuring blood levels of the amino acid homocysteine, but the patent also covers use of the test to infer levels of vitamins B-12 and B-6, which help break down homocysteine. In 1999, Metabolite and another company sued Laboratory Corp.—called LabCorp—for patent infringement and breach of contract. A

jury found LabCorp guilty of both offenses and awarded Metabolite $4.7 million in damages. The Federal Circuit upheld the judgment on appeal, further adding that doctors who use homocysteine levels to deduce vitamin B levels, regardless of the method they use, "directly infringe" Metabolite's patent each time they order the test and interpret the medical implications.

LabCorp argues that the relationship between homocysteine and vitamin B is a natural phenomenon that should not be patented. Many patent lawyers expect the high court to strike down that part of the patent, but they fear that the court may also scale back the kinds of things that can be patented. They divine the court's intention from its willingness to review LabCorp's submitted argument that the patent's assaying step was "indefinite, undescribed, and nonenabling" and the court's questions submitted to the U.S. solicitor general last year on whether Metabolite's patent should have been "invalid because one cannot patent 'laws of nature, natural phenomena, and abstract ideas.' "

Physicians believe the assaying-correlating step of the patent is invalid and fear that judicial approval of such a step would open the floodgates to other spurious patents. Such patents "operate to chill, not to promote, the progress of science … [and] the sound practice of medicine," wrote a coalition of medical societies, including the American Medical Association, in an amicus curiae brief to the high court. In another brief, Affymetrix, the Santa Clara, California–based gene array maker, urges the court to strike down the correlating part. Metabolite's brief warns that the wrong decision could disqualify drug patents because it would mean the inventors "merely discovered that certain chemicals interact with the human body in ways directed by chemistry."

Although many attorneys think that examiners went too far in awarding a patent on the correlating step to Metabolite, they worry that any corrective action by the high court will set a new and restrictive precedent. That could upend Federal Circuit decisions allowing patents on business methods, software, or biotechnology not spelled out in the aged patent laws. That would be a mistake, argues Hal Wegner of Foley and Lardner LLP in Washington, D.C., noting in a recent essay that future technologies "require an open door to patent eligibility" to win backing from Wall Street.

Parts of the biotech industry would be likely to feel the pinch from a restrictive court ruling, says Norviel. A growing roster of firms rely on test-plus-correlate claims for gene expression tests, including Genomic Health in Redwood City, California. Another is those that have developed methods of detecting cancer or other diseases. Such innovations won't be developed "if that sort of relationship is not patentable," says Norviel.

The power of a patent

One week after LabCorp, the justices will hear a case that tests whether a company found to infringe another's patent should automatically be stopped from further use of that patent. The eBay case pits the $55-billion-a-year online auctioneer against MercExchange, a small Virginia company that had patented some online auction methods beginning in 1998 but failed to commercialize them.

In 2003, a jury found eBay to be infringing on a patent for an auction method and awarded MercExchange $35 million. Such a ruling usually leads to a court-mandated prohibition of the use of an invention by the infringer, a disruption that would have cost eBay much more than the damages levied by the jury. But the court denied MercExchange's request for an injunction, citing a 1954 law that allows courts to reject requests for injunctions if they do not follow "the principles of equity."

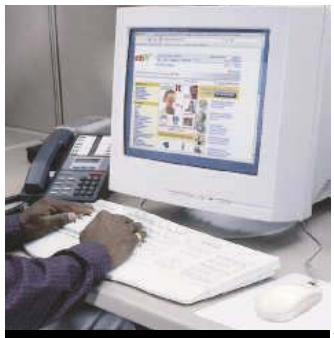
Early last year, the Federal Circuit Court disagreed and said the lower court should have given MercExchange the injunction. Such injunctions should be the "general rule," it opined, barring an "exceptional" public need such as a medical emergency. That ruling divided the high-tech community. Biopharma attorneys welcomed the decision, noting that the near certainty of an injunction is a big disincentive for generic drugmakers to go into production if there is a chance they may be caught infringing a patent. But communication and software lawyers—including those who represent RIM, the Canadian maker of the popular Blackberry e-mail device now facing patent woes—say that the threat of an automatic injunction gives too much leverage to companies seeking licensing fees for insignificant patents that they have acquired on the open market. They would like the courts to limit injunctions on behalf of these so-called patent trolls. The same issue scuttled the negotiations last year on the House reform bill.

In its brief, eBay argues that the Federal Circuit has overstepped its authority. A 1908 Supreme Court ruling that a company need not commercialize an invention to receive an injunction, it said, should be overturned if it "precludes equitable discretion." If the high court agrees, say some patent experts, university researchers may find it harder to collect licensing revenue from their inventions if they don't commercialize them. Also, says Noonan, "Small biotech Davids need injunctions to fight big company Goliaths."

Advocates who believe the patent system needs tweaks but not wholesale changes hope the high court treads lightly in these fundamental areas. Richard Taranto of Farr and Taranto in Washington, D.C., forecasts "modesty" by the court in deference to its inexperience. But Joshua Sarnoff of Washington College of Law at American University in Washington, D.C., speculates that the court may be interested in a major reform of the legal framework the Federal Circuit has created. He cites the fact that the high court ignored pleas by the U.S. government not to take the LabCorp case. Yet patent attorneys are also mindful of a 2002 decision involving cylinder manufacturers in which the high court expanded the rights of patent holders by reversing the Federal Circuit on an issue related to similar inventions.

The justices are expected to rule on both cases before the end of their current term in June. But that may not be their final word on patents. This spring, the high court is expected to decide whether to accept a case involving yet another fundamental patent issue: how high to set the obviousness bar for inventions. That can be a crucial factor in determining whether a new technology receives a patent. Fans of the status quo hope the court decides not to take the case, involving brake mechanisms. If it does, arguments will be heard in the fall.

–ELI KINTISCH



LabCorp v. Metabolite	*eBay v. MercExchange*
Originally filed: 1999	**Originally filed:** 2001
Oral arguments: 21 March	**Oral arguments:** 29 March
At issue: Patenting scientific principles	**At issue:** Power of a patent holder to halt an infringer's business

Setting the bar. The U.S. patent community is abuzz over a pair of cases to be heard next month by the Supreme Court.

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SCIENCE & ENGINEERING

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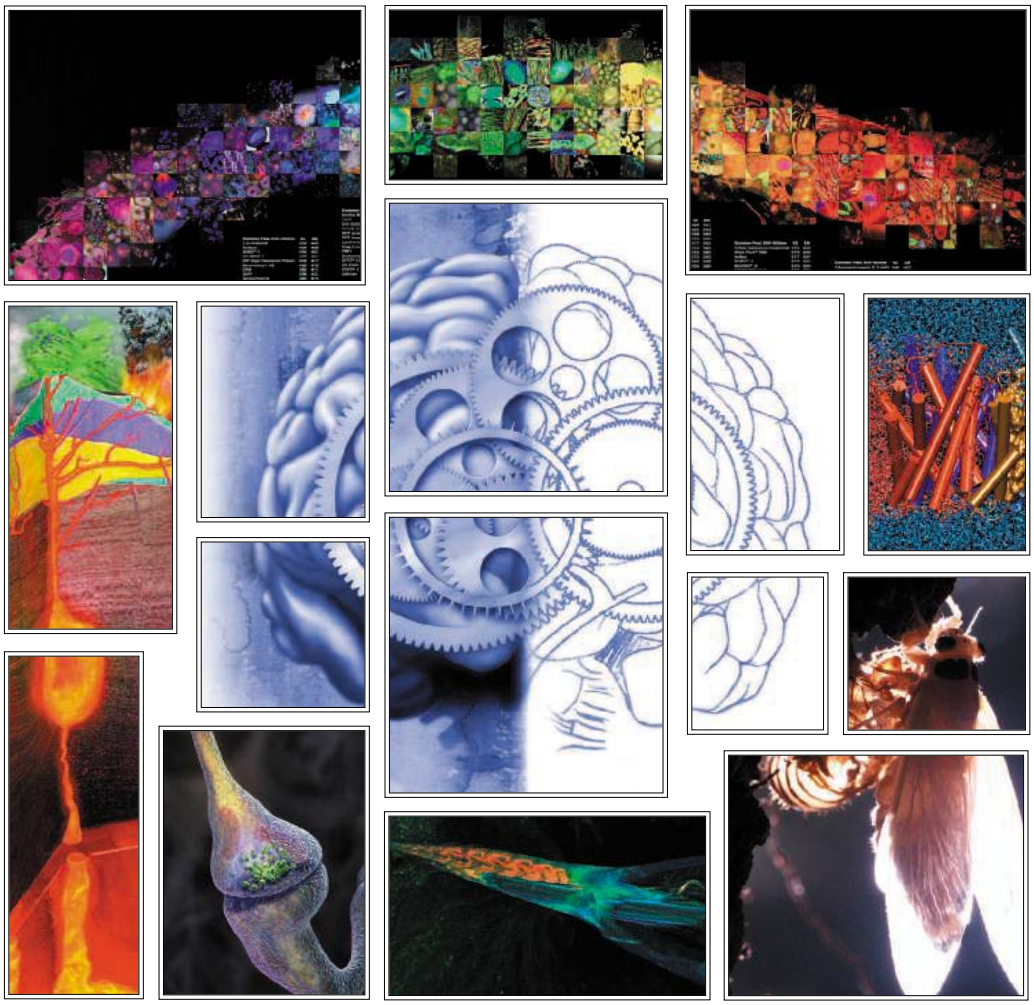

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EDITED BY YUDHIJIT BHATTACHARJEE

Movers

CHANGING SIDES. The latest official to spin through Washington's revolving door is Lester Crawford, a veteran Food and Drug Administration official who left FDA last fall after 2 months as commissioner. The 67-year-old Crawford, who cited his age as the reason for retiring, last month joined a Washington, D.C., lobbying firm, Policy Directions Inc., as senior counsel. The firm's recent clients include Merck, the National Association for Biomedical Research, and PhRMA, an industry group.

"This is, at the least, ethically a serious conflict of interest," says Sidney Wolfe of Public Citizen, a Washington, D.C., nonprofit and frequent critic of FDA. Crawford won't be lobbying the agency, the firm says, and will therefore stay on the right side of a federal law that prevents such activity by ex-administrators for a year after quitting government. "A lobbyist can still be effective without lobbying his former colleagues" directly, says Massie Ritsch of the Washington, D.C.–based Center for Responsive Politics.

POLITICS

POLICY KING. A Canadian banker turned policy guru has been named the first president of the Canadian Academies of Science (CAS).

Peter Nicholson, 63 (below), lost his job last month as a top adviser to Liberal Prime Minister Paul Martin after Canadian voters replaced Martin with Conservative Stephen Harper. Nicholson's diverse experience "inside and outside government" makes him an obvious choice for the job, says CAS Chair Howard Alper.

Royal Society past president William Leiss, who campaigned for a decade for the creation of the academies (*Science*, 22 October 2004, p. 589), is disappointed that CAS didn't pick somebody from among the 2200 elected members of its three founding organizations: the Royal Society, the Canadian Academy of Engineering, and the Canadian Institute of Advanced Medicine. "This is Canada doing its own thing once again," he says.

Nicholson hopes to silence such critics with his performance. "My approach to public service has been pretty nonpartisan," he asserts. He'll have plenty of opportunity to demonstrate that: A stated goal of CAS is to crank out 50 reports over the next 10 years on a variety of issues to be selected by the government.

MONEY MATTERS

PATRON SAINT. Johns Hopkins University in Baltimore, Maryland, this month received $100 million for stem cell research and other campus initiatives from an anonymous donor thought to be Michael Bloomberg, the billionaire mayor of New York City. The former chair of the university's board of trustees and a 1964 graduate of the school, Bloomberg has publicly given Hopkins $107 million in previous years and similar amounts anonymously, according to *The Baltimore Sun*.

Bloomberg has not denied news reports identifying him as the man behind the donation. Johns Hopkins spokesperson Dennis O'Shea won't confirm or deny that the gift comes from him. But he says "it is very clear that Mayor Bloomberg considers public health issues extremely important."

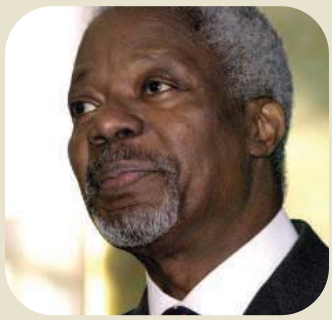
MOVERS

GLOBAL IMPACT. The Bill & Melinda Gates Foundation has picked a senior drug industry researcher to lead its efforts to bring new drugs and vaccines to the developing world. Tadataka Yamada, 60, chair of research and development at GlaxoSmithKline (GSK) since 2001, will become executive director of the $28.8 billion foundation's global health program in June. He replaces Richard Klausner, who left in December to head a new venture capital fund in Seattle, Washington.

A physician and former chair of internal medicine at the University of Michigan Medical School, Yamada tried to make GSK's research more efficient by organizing scientists around a half-dozen disease-specific teams. His background makes him well suited to help the foundation unite academic researchers and industry behind treatments for neglected diseases, says Seth Berkley, president of the International AIDS Vaccine Initiative.

Awards >>

ENVIRONMENTAL SYNERGY. United Nations Secretary-General Kofi Annan hopes to turn a $500,000 prize for environmental leadership into a foundation to promote agriculture and women's education in Africa. Last week, Annan received half of the Zayed Prize, funded by the crown prince of Dubai, for launching the Millennium Ecosystem Assessment project (*Science*, 1 April 2005, p. 41). A $300,000 slice of the biennial $1 million prize went to the global assessment project itself, and the remaining $200,000 to panel co-chairs Angela Cropper and Emil Salim, the former Indonesian environmental minister.



Annan first emerged as an environment champion at the 2002 world summit on sustainable development in Johannesburg, South Africa. He has stood out among environment advocates for his focus on "the link between ecosystems and long-term economic health," says James Sniffen of the U.N. Environment Programme in New York City.

Previous Zayed winners include former U.S. president Jimmy Carter and Nigerian climate scientist Godwin Obasi, co-founder of the Intergovernmental Panel on Climate Change.

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LETTERS

edited by Etta Kavanagh

The Language of Fighting Invasive Species

IN HIS ARTICLE "WINNING THE WAR AGAINST ISLAND INVADERS" (NEWS Focus, 2 Dec. 2005, p. 1410), K. Krajick presents an interesting point of view about eradication of exotic species on islands. However, despite the fact that exotic species are a leading cause of biodiversity loss, there is growing concern about the language (*1*) and the attitude (*2*) used by researchers in this area, since they may not be the most appropriate ways to capture the attention and support of laypeople. Gobster argues that the language used is too aggressive and strategies, such as eradication, are too brutal, pushing some organizations to stand against programs controlling exotic species. Public support is a fundamental part of any successful program dealing with exotics, and it has been shown that taking account of invader impacts is an important axis of biological conservation (*3*, *4*).

Biological invasions are complex: Interventions against rats that established on islands several centuries ago may require some discussions on possible nontarget effects and methods, but waiting to see the impact of a recently introduced species before attempting eradication is a crime against ecosystems, given current knowledge on the impact of alien species (*5*). The challenge today is educating the public on the negative effects of exotic species to obtain their support. Reducing the strength of the language used could increase the support of some groups, together with education to help them understand that, given their effects and the difficulties in predicting the success of exotic species, eradication is in some cases the only logical solution.

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Feral pigs roaming on Santa Cruz Island. A program is currently under way to eradicate this invasive species from the island.

CREDIT: AP PHOTO/NATIONAL PARK SERVICE

IN HIS NEWS FOCUS ARTICLE, "WINNING THE war against island invaders" (2 Dec. 2005, p. 1410), K. Krajick makes extensive use of the metaphor of warfare in his description of recent attempts to eradicate invasive species such as pigs and goats from island ecosystems. He writes, for instance, of the "war on pigs" on Santa Cruz Island and of a "war room" where professional hunters—no, "terminators"—gather to strategize. Ironically, though, some radical environmental activists who condone ecosabotage also like to use the language of warfare. Humans, they say, are prosecuting an aggressive war against defenseless nonhuman nature. Krajick ought therefore to be more circumspect in his choice of words. Why play into the hands of those who think of themselves as "eco-warriors," or of those in the animal rights movement who share their lack of compunction about property destruction? Krajick's description of an eradication program as an attempt by hired "terminators" to go after "the enemy" in hopes of "wiping out every last invader" will do nothing to help those who take animal welfare issues seriously (as we all should) to understand the ecological considerations that justify such programs. Since humans introduced the pigs to Santa Cruz Island, it makes sense to describe the eradication program simply as an attempt to rectify an earlier ecological mistake, rather than as a war against the pigs.

DEREK TURNER AND MICHAEL PATTERSON

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Doing More for Keisha

IN THEIR EDITORIAL "DOING MORE FOR KATE" (16 Dec. 2005, p. 1741), T. Cech and D. Kennedy describe a young woman who might have been a productive scientist, but was transformed into a business major by the anomie of a large research university. Keisha, a valedictorian from a working class high school, was also overwhelmed at a large research university. Rather than changing majors, however, Keisha transferred to a smaller public comprehensive university. There she was soon noticed by an instructor, who drew out her story and introduced her to other faculty. She is now engaged in an undergraduate research project, is academically successful, and seems well on the way to graduate school and a scientific career.

Although efforts to energize teaching in large classes should be applauded, it is a mistake to believe that this is more than a palliative for the real pathology that afflicts the U.S. scientific pipeline. The anecdotes about Kate and Keisha really say that scientific training and the development of a scientific workforce are intensely personal and human activities. This is not the mission of large research universities, nor are teachers of even the best large classes particularly good at it.

Assuming that intellectual capability in the young is not determined solely by the income of their parents, perhaps the greatest unexploited pool of scientific talent exists among the children of the working class. It is the public comprehensive universities, those invisible and disregarded institutions, that are most likely and best qualified to mentor and develop this impecunious human resource. Yet it is precisely those institutions that are being starved to death by state legislatures nationwide.

If Cech and Kennedy are correct that the U.S. scientific enterprise is threatened, then the science establishment might do even more for Kate and Keisha by attacking the political disease rather than the pedagogical symptoms.

WILLIAM S. BARNES

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Genetic Research into Autism

ACCORDING TO THE EXTREME MALE BRAIN (EMB) theory ("Sex differences in the brain: implications for explaining autism," S. Baron-Cohen *et al.*, Viewpoint, 4 Nov. 2005, p. 819), autism is characterized by extreme scores on two dimensions, empathizing (low) and systemizing (high), typical of a very masculine brain. Baron-Cohen *et al*.'s account omits an important part of the causal story: Autism is perhaps the most highly heritable behavioral disorder. Data from our recent twin study speak to the genetic architecture of autistic-like dimensions. The model of two (or more) dimensions underpinning autistic behaviors may be critical for understanding the causes of autism. This is because low correlations exist between social impairments (related to empathizing) and nonsocial behaviors like restricted repetitive behaviors and interests (related to systemizing) in the general population (*1*). Moreover, social and nonsocial behaviors are, like autism, both highly heritable, but largely genetically distinct, both in the general population and at the extremes (*2*). Low genetic correlations suggest that different genes contribute to social and nonsocial autistic traits. However, there is no evidence from twin studies of sex-specific genetic effects, only mean sex differences (well-documented in the article) and an increasing male bias toward the impaired extreme (*2*, *3*). The genetic mechanisms underlying sex differences in relation to fetal testosterone and autism still need to be mapped.

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Response

WE THANK RONALD *ET AL.* FOR HIGHLIGHTING the need for more genetic research into autism and agree that genes are likely to play a major role in the cause of autism spectrum conditions. It is too early to say whether the relevant genes are sex-linked, sex-limited, sex-influenced, or sex-independent. Although we do refer to genetics on p. 822, because of the strict word limit, we confined our Viewpoint to topics with sufficient evidence for sex-related effects relevant to both the empathizing-systemizing (E-S) and EMB theories. Sex-related effects relevant to the E-S and EMB theories are clear at the psychological level and suggestive at the neuroanatomical level. More work is needed to clarify the role of fetal endocrinology. At present, there is insufficient evidence to identify candidate genes that could give rise both to sex differences in empathizing and systemizing for the general population, and to a hypermasculinization of these in individuals with autism spectrum conditions (*1*).

Nevertheless, we agree with Ronald *et al.* that psychological, neuroanatomical, and endocrine effects are likely to be downstream of genetic antecedents. We can imagine that a gene such as the androgen receptor (AR) gene on the X chromosome would be a candidate in the EMB model, where variations in the CAG repeat length may be associated with phenotypic variation between the sexes (*2*) and between autism and controls.

Ronald *et al.* (*3*) found that social (or what we call E, for empathizing) and nonsocial (or S, for systemizing) traits are largely independent in a large normative twin sample. In our own studies, we too found that E and S are weakly but significantly negatively correlated in a normative sample ($r = -0.09$), although in an autism spectrum sample, the correlation is stronger and still negative ($r = -0.29$) (*4*). This may be consistent with Ronald *et al.* (*3*) in that they observed "All extreme groups had a higher proportion of males than females." So we might hypothesize that the low-E and high-S aspects of autism, although largely independent in the general population, both bear some permissive (although not determinative) relation to maleness when they occur to extreme degrees.

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Acid Growth and Plant Development

IN HIS PERSPECTIVE "GROWTH BY AUXIN: WHEN a weed needs acid" (7 Oct. 2005, p. 60), M. Grebe asserts that the acid-growth theory describes how the plant hormone auxin (indole-3-acetic acid, IAA) stimulates cell elongation in developing organs such as leaves and roots. On the basis of this statement, he presents the hypothesis that the proton pump AVP1 of the model plant *Arabidopsis thaliana* causes cell elongation via IAA-induced acid secretion. Grebe refers to a single review article (*1*) by Achim Hager, the spiritual father of the acid-growth concept. Hager's wall acidification model is based on experiments with shoots of grass seedlings (coleoptiles, which are leaf-like axial organs). The hypothesis was proposed in 1971 and thereafter carefully evaluated by scientists (*1*–*4*).

The observations that (i) acid buffers (pH 3.5 to 4.0) elicit a rapid short-term growth response in oat coleoptiles, (ii) IAA enhances the rate of proton extrusion so that a pH of about 5.0 is established in the growth-limiting organ walls, and (iii) metabolic inhibitors block both hormone-mediated wall acidification and cell elongation led to the postulate that auxin may initiate coleoptile elongation by rapidly lowering the apoplastic pH value from about 6.0 to about 4.8 to 5.0 (*1*). However, the fungal phytotoxin fusicoccin (FC), but not the naturally occurring growth hormone IAA, fulfils the predictions of the acid-growth hypothesis of coleoptile elongation (*2*). FC rapidly activates a plasma membrane–localized proton pump fueled by adenosine triphosphate (P-

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

ATPase) and thereby causes an acid efflux into the walls (final apoplastic pH about 3.5 to 4.0). The resulting burst of organ elongation is accompanied by a rapid rise in the rate of cell respiration. These results are in accordance with the acid-growth hypothesis of FC action, which has become a well-supported theory (*3*). Corresponding experiments with IAA (and FC, used as a tool) led to the conclusion that auxin-induced proton secretion is insufficient to elicit growth: When a wall pH of 4.8 to 5.0 was established by application of a suboptimal concentration of FC, no promotion of coleoptile elongation occurred (*2*). Alternative concepts of IAA-mediated cell-wall expansion have been proposed that are currently under investigation (*3*, *5*).

In addition, Grebe states that the wall acidification hypothesis of elongation growth also applies to roots, as implicitly assumed by Hager (*1*). This is not the case (*4*). In developing roots of grass seedlings, no positive acid (pH 4.0)–growth response has been recorded; at pH 3.5, organ elongation is reduced. In root cells, IAA inhibits rather than promotes the rate of proton secretion (*4*).

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Response

KUTSCHERA COMMENTS ON THE PERSPECTIVE I wrote discussing a study by J. Li *et al.* ("*Arabidopsis* H^+-PPase AVP1 regulates auxin-mediated organ development," Reports, 7 Oct. 2005, p. 121). This study provides the first functional genetic evidence that a plant proton pump, the pyrophosphate-driven *Arabidopsis* V-type ATPase AVP1, is required for auxin transport, cell wall acidification, organ growth, and development. Kutschera incorrectly states that I proposed that "the acid-growth theory describes how the plant hormone auxin (indole-3-acetic acid, IAA) stimulates cell elongation in developing organs such as leaves and roots." I simply posed the question of whether AVP1-mediated regulation of cell wall pH in roots or leaves involves an acid-growth mechanism similar to the one discussed by Hager (*1*). Additionally, I asked whether AVP1-dependent cell wall acidification may feed back on auxin transport, and whether AVP1 regulates intracellular transport of auxin carriers and plasma membrane H^+-ATPase (P-ATPase).

My Perspective has touched off an unsettled controversy among plant physiologists, as it remains a matter of debate as to what extent auxin-induced cell wall acidification contributes to elongation growth (*1*–*5*). Studies on several plant species experimentally support Hager's acid-growth theory (*1*, *3*), originally proposed for stems of grass seedlings (*1*, *4*). Other scientists, including Kutschera, have published evidence questioning the theory (*2*, *5*).

Given the tools and experimental approaches available at the time, most early studies addressing acid-growth employed external application of IAA and/or the fungal phytotoxin fusicoccin (FC), which plants do not produce endogenously (*1*–*5*). Kutschera correctly points out that FC stimulates cell wall acidification more efficiently than externally applied auxin (*2*). However, exogenous application of auxin and FC alone cannot determine whether plants intrinsically utilize auxin-dependent cell wall acidification to regulate growth processes.

Li and colleagues focus their discussion of AVP1 function on auxin transport regulation, taking into account that the classic chemiosmotic model of auxin transport has been

strongly supported by genetic and molecular studies (*6*). Similar to the work by Li and colleagues, genetic loss- and gain-of-function studies of endogenous proteins required for cell wall acidification and auxin responses may now be combined with physiological experiments to address the relevance of auxin-induced acid growth in planta. Studies of acid-growth responses in *Arabidopsis* seedling stems (hypocotyls) have been initiated (*7*). Similarly, auxin-stimulated acid growth of tomato hypocotyls as well as its loss in the *diageotropica* mutant has been reported (*7*, *8*). External application of auxin at high concentrations stimulates hypocotyl (*7*) but inhibits root elongation in *Arabidopsis* and tomato (*8*, *9*), consistent with experiments cited by Kutschera. Intriguingly though, application of external auxin at low concentrations stimulates *Arabidopsis* root growth (*9*). These findings raise the question of which growth responses are physiological processes regulated by auxin within the range of its endogenous concentrations. Thus, it remains to be revealed exactly where auxin-regulated cell wall acidification constitutes an intrinsic mechanism regulating different aspects of plant growth.

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CORRECTIONS AND CLARIFICATIONS

Random Samples: "Asian science on the move" (27 Jan., p. 447). The worldwide gross expenditure on research and development was reported wrongly. It comes to $830 billion and not $2.8 trillion as stated.

Reports: "Restoration of auditory nerve synapses in cats by cochlear implants" by D. K. Ryugo *et al.* (2 Dec. 2005, p. 1490). On page 1490, the number of nonexperimental subjects conflicts with those listed in the Supporting Online Materials. The SOM is correct: Six congenitally deaf cats and four normal hearing cats were used as controls.

Research Articles: "Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes" by B. P. Tu *et al.* (18 Nov. 2005, p. 1152). Several references were cited in the wrong places in the text. The correct sentences and citations are as follows: "A recent study described a ~40-min respiratory oscillation that produces a genome-wide, low-amplitude oscillation of transcription during continuous culture (*11*, *12*)… About two-thirds of the most periodic transcripts in the YMC encode components of mitochondria (Table 2) (*14*)… Might it be that an ancestral symbiont (*35*), endowed with the capacity to generate ATP by both respiratory and reductive pathways, used the two pathways in an oscillatory manner (Fig. 7B)?… Temporal compartmentalization of metabolic function also appears to take place during the circadian cycle of flies and mice (*27*, *28*). The primitive cyanobacterium *Synechococcus elongatus*, which conducts both photosynthesis and nitrogen fixation, uses its circadian regulatory apparatus to ensure that these biochemically incompatible pathways are executed at temporally distinct phases of the circadian cycle (*36*). The circadian cycle drives the periodic expression of many genes encoding the rate-limiting enzymes of numerous metabolic processes (*27*, *28*). Restricted feeding can entrain the circadian cycle (*35*, *36*), perhaps through metabolic feedback impinging directly on the transcription factors that themselves regulate circadian rhythm (*39*, *40*)."

Reports: "Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite" by M. Platten *et al.* (4 Nov. 2005, p. 850). The hydroxyl group on the benzene ring at the 3′ position on 3,4-DAA (anthranilic acid) in Fig. 1A should not be there.

METROPOLITAN GEOGRAPHIES

When Suburbs Collide

Jennifer Wolch

According to myth, the urban sprawl of Los Angeles and New York City will one day meet in a Nebraska cornfield. In *Sprawl*, Robert Bruegmann tells us not to worry about such an eventuality. He defines sprawl as "low density, scattered, urban development without systematic large-scale or regional public land-use planning." The book is a lively discussion about why our current preoccupation with sprawl is unnecessary and why policies to address it are misguided.

Bruegmann (a professor of art history, architecture, and urban planning at the University of Illinois at Chicago) argues that sprawl has always been with us and that attempts to cast sprawl in a negative light are wrongheaded. He claims that remedies for sprawl have not produced the intended effects and that toying with urbanization patterns created by millions of individual decisions is a risky business. Debunking widely accepted myths, *Sprawl* defends urban development as a largely beneficial expression of human desires that transcend geography and history. A welcome tonic to anti-sprawl advocacy, the book forces us to rethink received wisdom. It will be widely read and debated by professionals and academics alike.

All the more unfortunate then, that so many of the book's arguments are wrong. Just like the "anti-sprawl campaigners" he takes to task, Bruegmann cuts corners in his quest to create a defense of sprawl. This is bad science, which can only lead to bad public policy.

Where does Bruegmann go wrong? One flaw is his attempt to avoid the problem by defining it away. He correctly argues that categories such as city, suburb, and exurb are fluid. What one generation decries as suburban sprawl the next sees as a target for historic preservation. But is exurbia sprawl? His answer is no. Although most people would regard as sprawl a "subdivision of two-acre ranchettes with mowed front lawns," he declares that an "old farmhouse on two acres where the husband commutes to a job in a central city but the family farms the land themselves" is not. According to Bruegmann, whether they farm or not is irrelevant, because agricultural surpluses mean we don't need exurban land for food production. And he believes that the rich, rather than hurting central cities as they decamp to the exurbs, will save the day: their downtown shopping allows older cities to retain some residents until they can be gentrified. This facile attempt to define away exurban sprawl and its effects is unconvincing in a country where, between 1970 and 1990, over half the new residential land consumed was in lots over ten acres and over 90 percent in lots of one or more acres.

Bruegmann next conflates form with process. He argues that something akin to sprawl has characterized urbanization since the birth of cities. Indeed, such transhistoricity of sprawl implies that it is "natural" and has adaptive benefits for human societies. But arguing that the expansion of Europe's medieval cities beyond their walls was driven by the same dynamics as the post–World War II suburbanization of American cities is misguided. Not only is the geographic scale of the "city" vastly different, the forces driving growth—external threats, technologies, resource bases, economic and social structures, land tenure patterns, institutions of governance, etc.—are dissimilar. The superficial similarity in form masks enormous differences underlying process. Moreover, today many cities are no longer organized around a single center. Instead, the hinterland and multiple subcenters appear to be replacing the center. Bruegmann thus imposes a simplistic timeless organicism on urbanism that misses critical differences in the forces shaping cities across time and place.

Sprawl
A Compact History
by Robert Bruegmann
University of Chicago Press, Chicago, 2005. 307 pp. $27.50, £17.50. ISBN 0-226-07690-3.

The author's use of scientific evidence is also flawed. For example, we know that white flight from central cities with growing African American populations helped fuel 20th-century suburbanization and defensive municipal incorporation. In combination with exclusionary zoning, it also helped create wealth for white homeowners and their children. But Bruegmann dismisses the role of race on the grounds that one city (Minneapolis) with a small minority population still experienced sprawl. Or consider low-density development, which demonstrably depletes developable land and promotes more rapid rural-to-urban land conversion. Bruegmann claims that this does not matter because totally developed land in the United States occupies no more than 5% of continental land area, which allows the entire nation to live at "suburban densities" within the 65,000 square miles of Wisconsin. Such nonsensical aggregate data ignore the locale-specific constraints that invariably make sprawl problematic.

Confronted by issues of sustainability, Bruegmann complains that the concept is "warm and fuzzy" and relies on discredited notions of carrying capacity. He expects technology to extend resources to meet rising global demand, and he disregards mounting scientific evidence of rapid ecosystem services depletion by cities. Sprawl may actually promote sustainability, he claims, because "[a]t low enough densities, most citizens would probably be able to generate, using wind, water, solar, and geothermal power sources, a great deal of the energy they need on their own land." But what about habitat loss? No need to worry, because "species extinction is still not well understood" and needs more research. Similarly, Bruegmann concedes that human activities play a role in global warming, but "what can or should be done by whom and at what cost is still very much in dispute." Sprawling cities and their auto-dependency may not contribute to global warming, so attention to urban greenhouse gas reduction is unnecessary. This tactic—denying scientific evidence or consensus—has today become a depressingly familiar ploy to derail vital discussions on public policy matters.

After reviewing efforts to manage sprawl, Bruegmann concludes that results have been mixed at best. Even the nation's largest smart growth experiment (Portland, Oregon) provides no "silver bullet in the fight against sprawl." Unlike many European nations, the United States lacks the stomach for comprehensive land use management policies and suffers unanticipated negative effects from those policies that have been implemented. More important, sprawl is so good for so many, Bruegmann argues, that a market-driven approach to urban development is best. But because his ideas about the genesis of sprawl and its impacts are based on such weak science, his policy conclusions are inevitably misguided and impractical. The sustainability challenges created by a century of sprawl—concentrated poverty, fiscal disparities and socioeconomic polarization, jobs-housing imbalance, pollution and human health risk, habitat loss and species endangerment—cannot be addressed by individuals, families, businesses, and governments independently trying to maximize their utility. Concerted collective action has shaped cities in the past and is again needed to confront urban sprawl. Such action must be guided by the best social and natural science we can muster—and this *Sprawl* fails to deliver.

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ECOLOGY

Land of the Lost

Paul L. Koch

Twilight of the Mammoths
Ice Age Extinctions and the Rewilding of America

by Paul S. Martin

University of California Press, Berkeley, CA, 2005. 270 pp. $29.95, £18.95. ISBN 0-520-23141-4. Organisms and Environments.

In *The Twilight of the Mammoths: Ice Age Extinctions and the Rewilding of America*, paleoecologist Paul Martin describes his career-spanning efforts to understand the extinction of most large animals from around the globe near the close of the last ice age. Just 50,000 years ago, terrestrial communities were dominated by unfamiliar species: mammoths, mastodons, giant ground sloths, car-sized glyptodonts, rhino-sized marsupials, giant kangaroos, gorilla-sized lemurs, and many, many others. For millions of years, continental mammals had a bimodal body size distribution, with many species of rodent-to-rabbit-sized (small) mammals and a second mode of deer-to-elephant-sized (large) animals. Everywhere except Africa, this larger mode has almost entirely vanished.

As Martin recounts in the book—which is part memoir, part detective story, and part a call to environmental activism—a convincing explanation for these extinctions proved elusive into the 1950s, though climate change was thought a likely culprit. But as radiocarbon analyses began to clarify the timing of extinctions, Martin and others noted two startling patterns that pointed to humans as the causative agent. Whereas earlier extinctions affecting mammals always took out a greater proportion of smaller species, the late Quaternary extinctions were unusually fatal for large, slow-breeding animals. Furthermore, "when viewed globally, near-time extinctions took place episodically, in a pattern not correlating with climatic change or any known factor other than the spread of our species. Extinctions followed prehistoric human colonizations in a 'deadly syncopation.'" Martin became the most forceful advocate of the overkill hypothesis, offering sophisticated models to explain how human predation could drive extinctions while authoring studies on environmental change and the diets of extinct species that cast doubt on climatic explanations.

Here, writing for a general audience, Martin doesn't dive into overkill models in rigorous detail. But he broadly advocates "blitzkrieg," an idea he first advanced in the 1970s. Under blitzkrieg, immediately after arriving in unpopulated country, human range expansion and population growth are fueled by intense predation on large game. High kill rates are possible because naïve prey don't respond to an unfamiliar threat—armed humans. Extinction occurs rapidly, within decades at a regional scale. Blitzkrieg offers solutions to two perceived problems with overkill. Prey naïveté explains why the magnitude of extinction was high in the Americas and Australia but low in Africa, the region of human origins. And because the extinction was so rapid, blitzkrieg explains why archaeological sites containing extinct animals are rare in the Americas and Australia.

Overkill has been debated for decades. Some archaeologists remain troubled by the lack of kill sites and have questions about when humans arrived in the Americas and Australia. Animals on continents full of voracious predators may not have been so naïve to a new predator. On the other hand, detailed studies of the selectivity of extinction and predator-prey simulations support overkill. And despite spectacular increases in knowledge of near-time environmental change, no climatic hypothesis has ever explained the timing or selectivity of extinction. Some details of the extinction may have been modulated by environmental change, but the vast weight of evidence indicates that without human impacts, there would have been no late Quaternary mass extinction. That said, blitzkrieg as applied to extinctions on continents has always been a bit of a Rube Goldberg device, and it has become increasingly frayed in recent years. My view is that the extinction looks like the work of an omnivorous hunter (not a large-game specialist). As human populations slowly grew (fueled by small, faster-breeding game that could be harvested without causing extinction), tasty, large prey would be hunted whenever encountered and ultimately eliminated.

CREDIT: HERMANN TRAPPMAN

Martin's prose is folksy and engaging. It is a treat to learn the personal and historical story behind his studies of overkill. Chapters on his work in the American Southwest offer a glimpse of the twists and turns of field-based research and show how much is gained by taking a holistic multidisciplinary approach. The author spent a good deal of time literally studying crap, but this dirty business paid off with a nuanced understanding of animal diets. Equally fascinating is his discussion of animal figurines found with fossils in some caves. The figurines were placed in the caves in the last few thousand years, long after the extinctions. They hint that "for whatever reason—a focus on the hunt, or simple fascination—our ancestors were strongly drawn not only to living large animals, but also to the remains of extinct ones." The book has some editorial flaws, but they are minor and don't detract from the broad arc of Martin's narrative.

Amazingly, overkill is not the most controversial topic discussed in the book. In the final chapters, Martin explores the implications of the late Quaternary extinction for understanding the modern world. He recounts evolutionary "ghost stories," tales of species (such as the California condor) whose ecology makes no sense until one recognizes that they had co-evolved with now-extinct partners. And what should be the target of restoration ecology? The impoverished ecosystems that Europeans found when they arrived in the Americas and Australia, or the communities rich in large animals that lived there for millions of years? If history is any guide, North America without wild horses is completely "unnatural," yet ecologists and conservation managers continue to treat horses as an invasive species. Martin's most daring proposal is "rewilding." He proposes establishment of Quaternary parks in North America and Eurasia, with horses, camels, elephants, and recently lost carnivores (lions and cheetahs). The idea has provoked a range of responses, from disbelief, to calls for more study, to test cases.

Paul Martin has made a career of challenging orthodoxy and in the process has revolutionized the way we understand our history as a species and the world that we have shaped. It's fitting that he ends the excellent *Twilight of the Mammoths* with another bold idea that may let the sun shine again on the lost world of large animals.

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INFORMATION SCIENCE

Cybertools and Archaeology

Dean R. Snow,[1]* Mark Gahegan,[2] C. Lee Giles,[3] Kenneth G. Hirth,[1] George R. Milner,[1] Prasenjit Mitra,[3] James Z. Wang[3]

In archeology and other historical sciences, diverse, widely distributed data include artifacts, notes, field logs, and other records. Future research requires that these archives be electronically accessible and user-friendly.

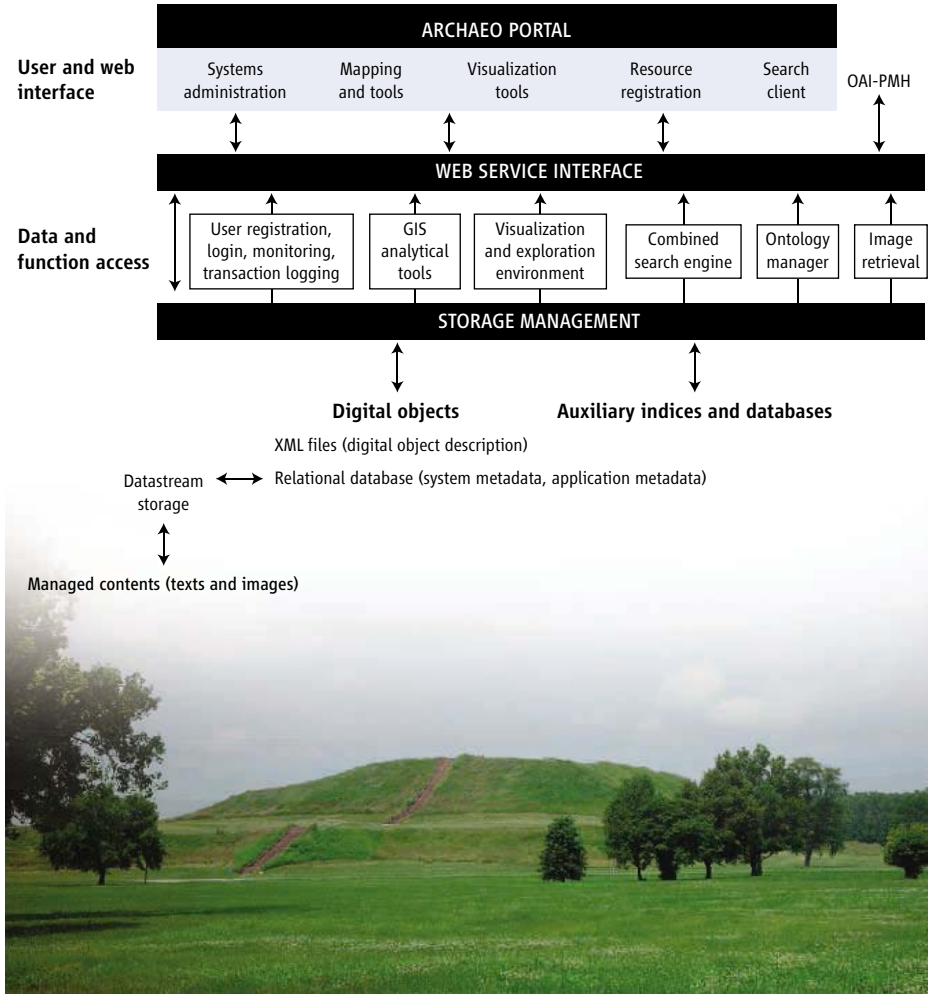
The need for service-oriented cyberinfrastructure (CI) has been reported (*1*–*4*). Further development of archiving and search tools to accommodate the explosive growth in many fields, particularly in biomedical research, has been emphasized. Archaeology often depends on archived data acquired by other researchers for other purposes, often long ago. Differences in recording protocols, terms, measurement units, and language are commonplace. Data are often obscurely archived and difficult to access, and policies regarding confidentiality vary considerably. Even when databases are accessible, they often differ in size, format, structure, and semantics and seem to defy fusion. In archaeology, research on the most important issues in today's society—the evolution of culture, the growth in population, and the long-term interaction of cultures with their physical and biological environments—will remain impoverished in the absence of a new generation of cybertools.

Enhanced online at www.sciencemag.org/cgi/content/full/311/5763/958

Modern archaeological science depends on large collections of diverse, mundane objects (such as potsherds, stone tools and debris, and animal and plant remains), rather than small collections of treasures. Sites are unique, nonrenewable resources easily destroyed by erosion or modern land use. Thus, old collections, original field notes, and reports of prior work have enduring research value.

At present, there are three types of data that are impossible to access simultaneously because of the highly individualized nature of traditional archaeological field and laboratory research. First, there are separately compiled databases held by museums, governmental agencies, and individuals that reside on different computer platforms. Data classifications and terminology vary, are regionally and temporally specific, and are inconsistently applied. Increasingly, these are Geographic Information System (GIS) databases based on years of accumulated paper records. Second, there is a voluminous unpublished "gray literature" consisting of limited distribution reports (produced mainly by cultural resource management firms and government agencies). Third, there are images, maps, and photographs embedded in museum catalogs and archaeological reports (published and unpublished). Difficulties in accessing data have been aggravated by the boom in cultural resource management (CRM) research in the United States. Government agencies, museums, universities, and private companies have acquired, and now care for, tens of millions of artifacts plus associated field notes and metadata.

The dimensions of the problem for the United States were outlined in a recent white paper commissioned by the Society for American Archaeology (SAA), in which it was estimated that six federal agencies alone require



Cyberinfrastructure architecture for archaeology. This proposed system integrates digital library middleware, document and image search, GIS analytical kits, visualization tools, and content management. The OAI-PMH (Open Archives Initiative Protocol for Metadata Harvesting) provides an application-independent interoperability framework for metadata harvesting by other repositories and similar systems. The architecture could be built completely from existing systems, some of which are open source. For example, Fedora is an open-source middleware for managing and serving digital objects and repositories. GeoTools is an open-source Java toolkit for producing interactive maps on the Web and GeoVISTA Studio is an open software development environment designed for geospatial data that allows users to quickly build applications for geocomputation and geographic visualization. SIMPLicity is a content-based image search and automatic learning-based linguistic indexing system. These are meant to be examples of software possibilities; no specific product endorsement is intended. Monk's Mound, Cahokia Mounds, Collinsville, IL, is pictured.

PHOTO CREDIT: D. R. SNOW

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~64 million cubic feet of collection storage space in addition to over 40,000 linear feet of documentation (*5*). These numbers do not include collections, images, and catalog documentation maintained by universities and museums (including the Smithsonian Institution) or state agencies across the country (*6*).

The problem is not only one of access. The various autonomous user communities currently hosting these resources understand and describe data in different ways. There is a need to classify and search for numerical, textual, and visual data simultaneously. Standards and protocols, where they exist, are typically defined at state or local levels and have changed over time. Diverse research interests and reliance on old collections require archaeologists first to understand each other's concepts and procedures in order to comprehend others' data, methods, and results. Translation protocols are needed. Regional data have often been collected and organized according to modern political boundaries that have no meaning whatsoever in prehistoric, early historic, or environmental contexts. Furthermore, some data, particularly precise site locations, must be kept confidential at levels that vary according to state and local policies. Consequently, archaeological research remains a mosaic of parochial efforts. Even the spatial and temporal dimensions of regional cultures must be extrapolated from selected sites and radiocarbon dates. Research on large geographical areas is particularly difficult at present.

Recent developments in computer and information science provide the computational tools, protocols, and standards that can help devise an integrated infrastructure. Federated databases and ontology-based database integration (*7*–*9*) provide the means of coordinated data management. Portal technology and Web services provide customizable access points to methods and data (*9*), and grid technology, coupled with high-speed data connections, provides distributed but high-powered computational resources (*10*). In several sciences, it is already apparent that a coordinated approach to describing, archiving, and disseminating research products and services can provide significant gains in productivity and quality. For example, the National Science Foundation (NSF) is currently funding the development of CI in support of the human-environment interaction and geoscience communities via the Human-Environment Regional Observatory Network (HERO) (*11*) and Geosciences Network (GEON) (*12*) projects. Within government, the National Map, the National Spatial Data Infrastructure (*13*), and Geospatial OneStop are federally mandated initiatives to improve sharing and collaboration among data collection agencies and users. A digital library and search engine for computer and information science, CiteSeer (*14*, *15*), is very popular with the computer science community. The SIMPLIcity (Semantics-sensitive Integrated Matching for Picture Libraries) content-based image retrieval engine has been used to manage large-scale databases for art and cultural, remote-sensing, biomedical, and Web applications (*16*). Readers can try it out online. Rather than try to impose a single formal data model and associated semantics on the community of researchers, new tools should instead take an approach that encompasses many different perspectives by developing database mediation services based on successful current approaches such as those used by GEON (*17*), the Fedora Digital Library (*18*), and ONION [Ontology Composition System (*19*)]. Thus, researchers should be able to query electronic data using search terms that have meaning to them, and these terms should be mapped to semantic equivalents when used to query remote data collections, then translated back. The CI architecture shown (see figure, page 958) is an example of an approach to facilitate use of archaeological resources via Web/grid services.

At the same time, any new system should facilitate future efforts within the archaeological community to establish common, minimal standards for metadata descriptions of artifacts, sites, maps, and other academic resources (*20*). Thus, interoperability is not simply a technical end-goal. It is instead a design strategy that also promotes effective cooperation between human and electronic components of the research process. Such efforts have begun at universities such as The Pennsylvania State University, Arizona State University, and the University of Arkansas and at the National Park Service. We require an e-science that marries the interconnectedness of digital research tools with the introspection enabled by traditional record-keeping (*21*, *22*).

Sustainability can be assured in two ways. First, data collections should be distributed and sharable. Host institutions should retain the freedom to manage their own databases for their own purposes, thereby spreading costs and maintaining institutional autonomy. Second, digital libraries and associated services should be made available to researchers and organizations to store their own data and mirror data of others. Because databases can remain in place, once the infrastructure is completed, the running costs would be restricted to maintenance and refinement of the metadata collections and associated services. Such a distributed system avoids trying to manage an ever-increasing centralized digital archive into the foreseeable future, with significant recurrent annual costs, but does bring up problems associated with federated search and management. Harvesting and indexing services can continue with minimum support and can be replicated by other organizations, so functionality can become truly distributed over time. The best system will be one that has commitments from government, academic, and commercial organizations.

Emerging cybertools can transform the way in which researchers collaborate to solve long-standing problems by providing: (i) a stable set of catalogs to preserve what is known about important data resources; (ii) tools to help researchers locate, access, and contribute data resources; and (iii) shared virtual workspaces in which researchers can collaborate virtually on larger tasks (see figure, page 958). Any attempt to impose a single complex (and expensive) system will fail. CI will be successful if it is allowed to evolve as it is adopted, used, and contributed to by a community. Encouraging archaeologists to do so also involves solving problems of confidentiality and trust, and securing long-term commitment from agencies.

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EVOLUTION

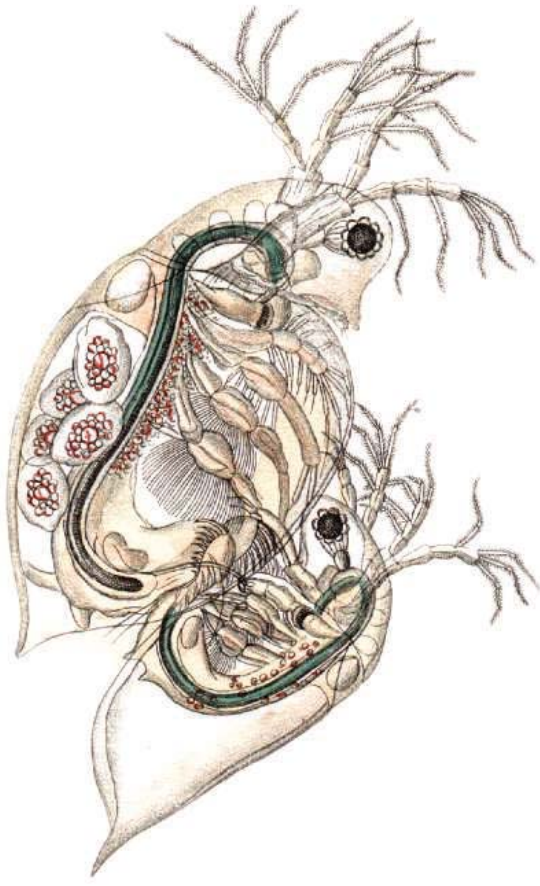
Why Sex?

Rasmus Nielsen

It is assumed that most organisms have sex because the resulting genetic recombination allows Darwinian selection to work better. It is now shown that in water fleas, recombination does lead to fewer deleterious mutations.

Why sex? This has been one of the most fundamental questions in evolutionary biology. In many species, males do not provide parental care to the offspring. Clearly, the rate of reproduction could be increased if all individuals were born as females and reproduced asexually without the need to mate with a male (parthenogenetic reproduction). Parthenogenetically reproducing females arising in a sexual population should have a twofold fitness advantage because they, on average, leave twice as many gene copies in the next generation. Nonetheless, sexual reproduction is ubiquitous in higher organisms. Why do all these species bother to have males, if males are associated with a reduction in fitness? The main solution that population geneticists have proposed to this conundrum is that sexual reproduction allows genetic recombination, and that genetic recombination is advantageous because it allows natural Darwinian selection to work more efficiently. New empirical evidence supporting this theory now comes from a study by Paland and Lynch on page 990 in this issue (*1*).

One reason why selection works more efficiently in the presence of recombination—that is, the exchange of genetic material between chromosomes—is that selected mutations tend to interfere with each other in the absence of recombination (*2*, *3*). Imagine, for example, a beneficial mutation (A) arising in one individual and another beneficial mutation (B) arising in another gene in an individual that does not carry mutation A. In the absence of recombination, mutation B would be eliminated when mutation A reaches a frequency of 100% in the population, and vice versa. No individual carrying both beneficial mutations could be created, and only one of the mutations could eventually reach a frequency of one in the population. Recombination speeds up the rate of adaptive evolution because it allows several beneficial mutations to be combined in the same individual. Likewise, when multiple deleterious mutations are present in the population, recombination has the potential for creating new offspring chromosomes with fewer deleterious mutations than either of the parental chromosomes. The famous population geneticist John Maynard-Smith compared this situation to having two cars: one with a broken engine and one with a broken transmission. Neither of them can run, but if you can replace the broken part in one car with a part from the other car you can produce a new functional car. Recombination allows broken parts to be shuffled among chromosomes, allowing new combinations to arise for selection to act on. Under suitable assumptions regarding the way deleterious mutations affect organismal fitness, the advantage of recombination in eliminating deleterious mutations can outweigh the twofold cost of sex (*3*).



Lessons from the flea. The water flea, *Daphnia pulex*, may shift from being sexual to being asexual and is, therefore, an excellent research tool for testing theories regarding the evolution of sexual reproduction. The drawing [from (*9*)] is by J. J. Paschoud in the late 1700s.

However, the selection theories are not free of contradictions and problems. Some of them rely on so-called group-selection arguments, where adaptive properties are properties of a whole population and not of individuals. If sexually reproducing individuals and their offspring do not have an immediate selective advantage in otherwise asexual populations, it is hard to see how populations can ever evolve from asexual to sexual reproduction. Additionally, the best explanations regarding deleterious mutations rely on strong assumptions regarding the distribution of selective effects (*3*), and there may be other factors favoring sex, such as increased resistance to pathogens (*4*). An observed genomic correlation between the rate of recombination and variability within species (*5*) suggests that there is an interaction between selection and recombination, but a direct difference between sexual and asexual populations has been hard to establish.

However, the new study by Paland and Lynch (*1*) provides direct empirical support for an excess accumulation of mutations in asexually reproducing populations compared to sexual populations. They examined different populations of the small crustacean *Daphnia pulex*, a type of water flea. *Daphnia* are excellent organisms to study in this regard because parthenogenetic *Daphnia* populations have arisen multiple times from sexual populations. Comparing asexual and sexual populations of *Daphnia* is, therefore, the perfect tool for examining the population genetic consequences of sexual reproduction (see the figure).

Paland and Lynch (*1*) compared the number of mutations with possible functional effects (nonsynonymous mutations) to the number of mutations with no functional effects (synonymous mutations) in 14 asexual and 14 sexual *Daphnia* populations. They observed a clear excess of nonsynonymous mutations in the asexual populations. They also estimated that close to 90% of the nonsynonymous mutations were subject to selection. These results suggest that the asexually reproducing species carry a higher load of deleterious mutations and that selection is not as efficient in the asexual as in the sexual populations. It does not directly demonstrate that selection against deleterious mutations is what maintains sexual reproduction, but the results do confirm the most important component of the selection theory: Asexual reproduction leads to an accumulation of deleterious mutations. It seems that males are allowed to exist after all, because they help females get rid of deleterious mutations.

The study is also interesting from another

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point of view. The estimate of the proportion of new mutations in *Daphnia* that are under selection is fairly high (>90%). Over the past 30 years, the paradigmatic theory in molecular evolution has been the Neutral Theory (*6*), which assumes that the vast majority of genetic polymorphisms have little or no selection acting upon them. However, the study by Paland and Lynch (*1*), and other recent studies (*7*, *8*), suggest instead that many or most polymorphisms may be under selection. Slowly, our weltanschauung in evolutionary biology is changing from a static view of a largely optimized genome to a dynamic view of organisms constantly challenged by selection and struggling with the large genetic load imposed by deleterious and new advantageous mutations segregating in the population.

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PLANETARY SCIENCE

Ringing the Changes

The Hubble Space Telescope has revealed two new moons and two new rings orbiting Uranus. The images indicate a dynamic, evolving, and possibly unstable ring system.

Carl D. Murray

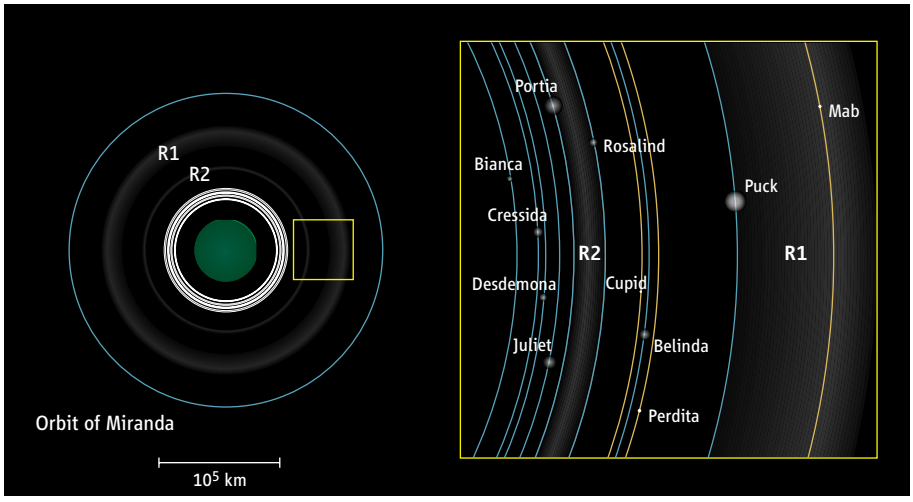
It all seemed so simple for a while. As far as we knew, Jupiter, Saturn, Uranus, and Neptune each had a ring system, a suite of small moons orbiting just beyond or close to the rings, followed by an impressive set of large moons and finally an irregular collection of outer moons in eccentric, inclined orbits. But now Uranus has yielded a few more secrets in the form of new moons and new rings. The moons may be tiny and the rings faint, but the research reported by Showalter and Lissauer (*1*) on page 973 of this issue reveals a complicated, evolving system that provides a fascinating insight into how rings and small moons are inextricably linked.

Of all the ringed planets, Uranus's ring system has seemed the most straightforward. It was made up of a series of dark, narrow, sharp-edged rings, the most unusual of which was the outermost ε ring complete with its two "shepherding satellites," Cordelia and Ophelia. Beyond the rings but still well within the orbit of Miranda (see the figure) lay an additional eight small moons discovered by the Voyager 2 spacecraft during its flyby in 1986. The largest and most distant of these was Puck, with an estimated diameter of 162 km. Karkoschka (*2*) later found an additional small moon, Perdita, lurking in the Voyager archive. Perdita orbits between the moons Belinda and Puck and is the outermost member of the "Portia group" of moons. In a series of numerical simulations, Duncan and Lissauer (*3*) had already shown that these moons were likely to be unstable, leading to a possible tragic climax of Shakespeareian proportions with Desdemona colliding with Cressida or Juliet within the next 4 million to 100 million years.

In their new work, Showalter and Lissauer have used sequences of images from the Hubble Space Telescope to show that the picture is not yet complete. Observations of small moons this close to a planet are notoriously difficult; long exposures are required, and the brightness of the planet tends to result in pictures with extensive "bloom" where saturated detector pixels engulf the image. However, by making use of the fact that the bloom on Hubble's High Resolution Camera is always in the vertical direction, the authors were able to align and time their images such that all the inner moons would show up in sufficiently long exposures. This led to the discovery of two new moons, Cupid and Mab, and two faint, diffuse rings that they refer to as R1 and R2.

The outer ring, R1, peaks in intensity at the orbit of Mab. This suggests that Mab is the source of the ring material, probably as a result of impacts from meteoroids or continuing collisions with the ring material already produced. Although the ejected particles can escape from the surface of the moon, they still have orbital energies comparable to their parent body and so remain in a broadly similar orbit. Jupiter, Saturn, and Neptune all have examples of small moons embedded in faint rings. There are still some anomalies in Mab's orbit that need to be understood, but the association with R1 seems clear. The Hubble observations have also shown that Puck, which orbits at the inner edge of R1, appears to have one side that is 10% brighter than the other. Puck, like our own and many other moons, is tidally locked to its planet and therefore has well-defined leading and trailing hemispheres. Given that it is Puck's trailing side that is brighter, the clear implication is that Puck's leading hemisphere has been darkened by impacts of material from R1.

The second new ring, R2, is neatly bounded by the orbits of two of the known moons, Portia and Rosalind. This suggests that some sort of



Inner region of Uranus system. (Left) The newly discovered rings R1 and R2 lie between the orbit of Miranda (the innermost of the large, regular satellites of Uranus) and the narrow rings detected by stellar occultation in 1977. **(Right)** A schematic close-up of the region near the new rings, showing the locations of the orbits of all satellites, including the recently discovered Cupid, Perdita, and Mab. The satellites are shown to their correct relative sizes (based on their mean radii), but these have been exaggerated by a factor of ~16 compared with the radial scale of the satellites' orbits.

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confinement mechanism is at work, because narrow, dusty rings such as these tend to spread as a result of nongravitational forces. Neither Perdita nor the comparably sized moon Cupid, the other moon discovered by Showalter and Lissauer, has an associated ring, but the authors point out that each lies close to the orbit of the larger moon Belinda, and in each case the moon's gravitational effect may well scatter any potential ring material. However, combining the Hubble images with the Voyager 2 data has allowed the authors to identify a unique property of Belinda and Perdita: They are likely to be locked in a gravitational resonance, whereby a simple numerical relationship between their orbital periods provides a mechanism that protects Perdita from collisions with Belinda. Whereas the Saturn system, for example, is riddled with resonances between moons, probably as a result of tidal evolution over the age of the solar system, this is the first example detected between two of Uranus's moons, large or small.

By comparing the current orbits of all of the moons in the Portia group with those derived more than a decade ago, Showalter and Lissauer provide clear evidence that the orbits are changing as the moons interact with one another. As a result of this work, the picture that emerges is one of a relatively young system of moons and rings that is still evolving in a chaotic fashion on time scales ranging from ~10 to ~10^7 years.

One of the strengths of planetary science is that it is comparative; nature has provided us with a set of parallel experiments (with different starting conditions) to study ring-satellite interactions around all of the giant planets, Saturn being the best example (*4*). Our task is to develop viable theories faster than the systems themselves are evolving.

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NEUROSCIENCE

SUMO Wrestles the Synapse

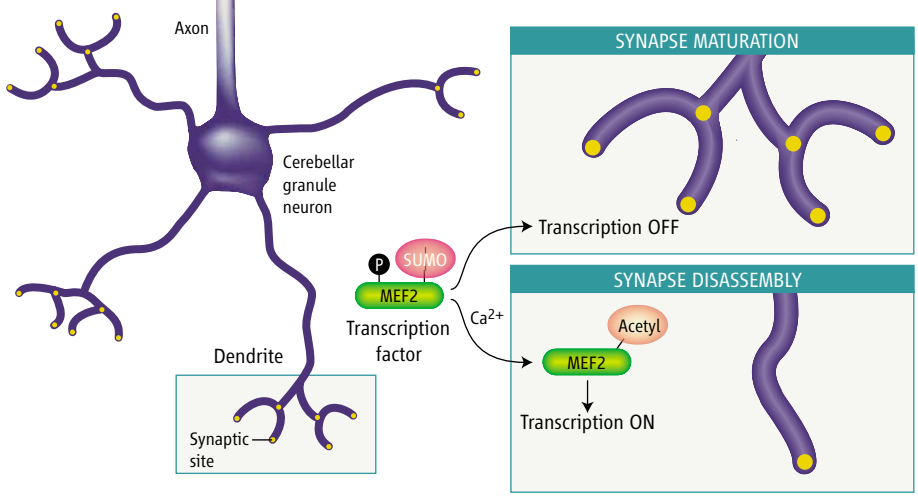
The number of synapses formed in the mammalian brain is controlled by electrical activity in neurons that drives calcium inward, regulating transcription factors, including one that causes removal of synapses.

Asim A. Beg and Peter Scheiffele

A single neuron in the mammalian brain forms thousands of specialized connections with other neurons called synapses. The number, strength, and specificity of these synaptic connections ultimately determine and regulate brain function. Consequently, one of the critical questions in neurobiology is how synaptic connectivity is established during development and how it is modified during life.

The activity of neurons has emerged as an important determinant in shaping neuronal networks. This might be best exemplified in the developing visual system by the activity-dependent segregation of inputs from one eye into specific domains that alternate with domains that receive information from the other eye. In principle, such activity-dependent mechanisms can regulate connectivity in two ways: by local changes in the cytoskeleton of individual neuronal processes that forge synapses, or by transcriptional changes in the cell nucleus (*1*, *2*). Calcium signaling plays a key role in eliciting neuronal activity–dependent transcription. For example, the growth of dendritic trees, the elaborate extensions on which a neuron receives most of its synapses, as well as the formation of synapses themselves, are stimulated by CREST (calcium-responsive transactivator) and CREB (cAMP response element–binding protein), two proteins that activate transcription in response to increased intracellular calcium (*3*–*5*). On pages 1008 and 1012 of this issue, two groups now report that they have uncovered an additional activity-dependent transcriptional program that controls synaptic differentiation in mammals through the myocyte enhancer factor 2 (MEF2) family of transcription factors (*6*, *7*).

Previous work had implicated MEF2 proteins in neuronal survival. To test for a requirement of MEF2 isoforms in synapse formation, Flavell and colleagues used RNA interference to suppress expression of MEF2A and MEF2D, the two major MEF2 isoforms in rat hippocampal neurons. Knockdown of their expression resulted in a doubling of the number of glutamatergic synapses formed, whereas overexpression of MEF2A led to a decrease (*6*). These alterations were dependent on the ability of the MEF2 proteins to stimulate neural activity–dependent transcription of target genes. In a second independent study, Shalizi and colleagues analyzed the role of the MEF2A isoform in cerebellar granule cells, another neuronal cell type of the brain (*7*). As in hippocampal neurons, MEF2A was required for normal synaptic differentiation. However, whereas loss of MEF2 isoforms in hippocampal neurons led to an increase in synapse number, loss of MEF2A in granule cells resulted in a decrease in synaptic structures. More specifi-



A synapse maturation switch. Sumoylation switches MEF2 function in cerebellar neurons from a transcriptional activator to transcriptional repressor. In the absence of calcium signaling, MEF2 is phosphorylated (P) and sumoylated (SUMO), represses expression of the transcription factor Nur77, and promotes synapse formation and maturation. Calcium influx triggered by neuronal activity causes dephosphorylation of MEF2 by the phosphatase calcineurin, resulting in desumoylation and acetylation (Acetyl). This allows MEF2 to activate target genes that promote synapse disassembly.

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cally, suppression of MEF2A by RNA interference prevented the formation of dendritic claws, the branched structures positioned at the tips of the short stunted granule cell dendrites. Because granule cells receive the majority of glutamatergic inputs on these dendritic claws, MEF2A-deficient cells receive fewer synaptic inputs than do MEF2A-expressing cells.

These seemingly opposite actions of MEF2 isoforms in hippocampal neurons and cerebellar granule cells are likely caused by differential regulation of MEF2 by two posttranslational modifications: phosphorylation and sumoylation (see the figure). First, both studies suggest a model whereby phosphorylation of a serine residue in MEF2 prevents activation of MEF2 target genes. Calcium influx through NMDA (*N*-methyl-D-aspartate) receptors as well as through voltage-gated calcium channels activates the phosphatase calcineurin, which in turn dephosphorylates MEF2. This then triggers MEF2-dependent transcription. The MEF2A target genes ultimately mediate synapse disassembly. Shalizi and colleagues suggest that in cerebellar granule neurons, MEF2A may primarily act as a transcriptional repressor. This functional switch from activator to repressor is controlled by a second posttranslational modification: sumoylation. In the phosphorylated form, MEF2A is further modified with a sumo subunit, an 11-kD polypeptide covalently attached to a lysine residue. Shalizi *et al.* suggest that phosphorylated and sumoylated MEF2A represses transcription of target genes and thereby promotes synaptic differentiation in cerebellar granule neurons. Conversely, either loss of MEF2A expression or MEF2A activity by its desumoylation (and subsequent acetylation) permits transcription of target genes and inhibits postsynaptic differentiation (see the figure). Shalizi *et al*.'s data suggest that MEF2A is not required for the activation of these synapse-disassembly genes in granule cells, only for their repression. This sumoylation switch might underlie the differential effect of MEF2 on synapse number in cerebellar and hippocampal neurons.

Both studies explored candidate genes that are directly regulated by MEF2. In cultured hippocampal neurons, transcriptionally active (dephosphorylated) MEF2 stimulates expression of SynGAP and Arc, two previously characterized neuronal signaling molecules. In cerebellar granule cells, sumoylated and phosphorylated MEF2A represses transcription of Nur77, which is itself a transcriptional regulator. It is noteworthy that all of these MEF2 targets act as inhibitors of synaptic differentiation, thereby providing a mechanism for the regulation of synapse number through the MEF2-dependent transcriptional program.

These two interesting studies add to earlier studies that explored mechanisms of calcium-dependent transcriptional regulation in developing neurons (*2*, *8*). The exciting new twist in this story is the identification of a calcium-dependent (and hence activity-dependent) transcriptional program that inhibits rather than promotes the formation of synapses. Future work should clarify how synapse-promoting and synapse-inhibiting gene expression programs are coordinated. One attractive hypothesis is that MEF2-dependent gene products pose a homeostatic constraint on activity-dependent synapse formation. Perhaps the MEF2-dependent synapse disassembly pathway balances signals that promote dendritic growth and synapse formation. Alternatively, different calcium-dependent transcriptional programs may be selectively activated depending on the pattern of neuronal activation and intracellular calcium concentrations. A similar mechanism has been previously observed for calcium signaling in different forms of synaptic plasticity (*9*). Finally, MEF2 target gene products may act differentially on existing synapses by preferentially destabilizing inactive synapses. Such a mechanism would be well suited for sculpting the connectivity pattern of the brain in response to sensory experience and might contribute to the extensive synapse elimination observed during postnatal life.

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ATMOSPHERIC SCIENCE

The Greenland Ice Sheet and Global Sea-Level Rise

Julian A. Dowdeswell

The flow of several large glaciers draining the Greenland Ice Sheet is accelerating. This change, combined with increased melting, suggests that existing estimates of future sea-level rise are too low.

The changing mass of the great ice sheets of Greenland and Antarctica represents the largest unknown in predictions of global sea-level rise over the coming decades. At 1.7 million km^2, up to 3 km thick, and a little smaller than Mexico, the Greenland Ice Sheet would raise global sea level by about 7 m if it melted completely. This could take from a millennium to a few thousand years (if melting were the only mechanism by which it lost mass) depending on the magnitude of future warming (*1*). Of more immediate concern are several sets of new observations, derived largely from remote-sensing satellites. As reported by Rignot and Kanagaratnam (*2*) on page 986 of this issue, the velocities of several large glaciers draining the ice sheet to the sea, already among the fastest-flowing on Earth, have recently doubled to reach over 12 km year^{-1}. In addition, the ice sheet has experienced a greater area of surface melting this year than at any time since systematic satellite monitoring began in 1979 (*3*). Both these changes increase mass loss from the ice sheet, with the implication that current estimates of global sea-level rise over the next century, of about 0.5 ± 0.4 m (*4*), may be underestimated.

The Greenland Ice Sheet gains mass through snowfall and loses it by surface melting and runoff to the sea, together with the production of icebergs and melting at the base of its floating ice tongues. The difference between these gains and losses is the mass balance; a negative balance contributes to global sea-level rise and vice versa. About half of the discharge from the ice sheet is through 12 fast-flowing outlet glaciers, most no more than 10 to 20 km across at their seaward margin, and each fed from a large interior basin of about 50,000 to 100,000 km^2. As a result, the mass balance of the ice sheet depends quite sensitively on the behavior of these outlet glaciers.

Two changes to these glaciers have been observed recently. First, the floating tongues or ice shelves of several outlet glaciers, each several hundred meters thick and extending up to tens of kilometers beyond the grounded glaciers, have broken up in the past few years (*5*). Second, measurements of ice velocity made with satellite radar interferometric methods have demonstrated that flow rates of these glaciers have approximately doubled over the past 5 years or so (*2*, *5*). The effect has been to discharge more

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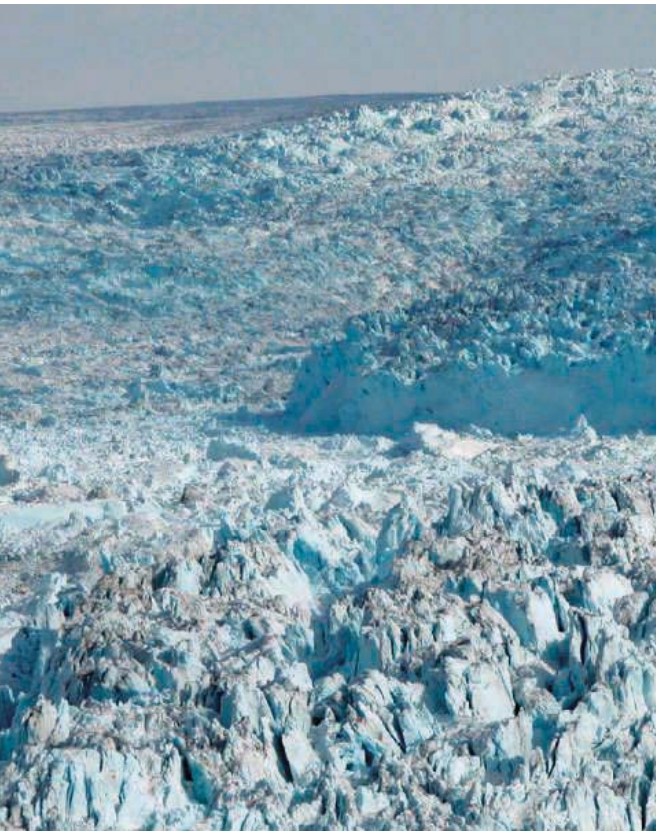
ice and, thus, to increase the mass deficit of the ice sheet from a little more than 50 km^3 $year^{-1}$ to in excess of 150 km^3 $year^{-1}$ (*2*). Increased velocity, combined with rapid dynamic thinning of up to 15 m $year^{-1}$ that cannot be accounted for by increased melting, may be linked to the loss of the mechanical buttressing effect of the ice tongues (*2*, *5*, *6*).

The outlet glaciers in question, including Jakobshavn Isbrae (see the first figure) in the west and Kangerdlugssuaq Glacier (see the second figure) on the east coast of Greenland, are all south of 70ºN, suggesting that there may be some linkage with changing climate. Satellite data from passive microwave instruments show that there has been a very marked increase in the area affected by summer melting and the length of the melt season on Greenland. Indeed, 2002 and 2005 are records for melt extent over the 27 years of observations (*3*). With these observations and a meteorological model to retrieve annual accumulation, runoff, and surface mass balance for the ice sheet, a declining mass balance over the past 6 years (to 2003) was calculated (*7*). Not only did this change in surface balance yield a contribution of 0.15 mm $year^{-1}$ to global sea-level rise, but it may also be implicated in the changing velocity structure of the ice sheet. Increased meltwater production, if it reaches the glacier bed through crevasses, provides a clear mechanism for enhanced flow through basal lubrication and sliding. A clue to this is provided by observations of the coincidence of ice acceleration with the duration of summer melting on the ice sheet even beyond the boundaries of fast-flowing outlet glaciers (*8*).

The breakup of marginal floating ice tongues in Greenland may also be related to the increase in meltwater production and its penetration into surface crevasses. A similar mechanism of hydromechanical fracturing was responsible for the disintegration of much larger fringing ice shelves on the Antarctic Peninsula (*9*). Importantly, breakup of these Antarctic ice shelves was linked to rising temperatures and was followed by velocity increases of between two and eight times (*10*).



Fast glacier flow. The margin of the fast-flowing outlet glacier Jakobshavn Isbrae, West Greenland. This glacier drains an interior basin of more than 90,000 km^2 (*2*). At a velocity of more than 12 km $year^{-1}$ (*5*), it is one of the most rapidly moving glaciers in the world. The glacier terminus is shown by the vertical ice cliff, which is up to about 100 m in height. Above this, the heavily crevassed surface of the outlet glacier can be seen. Below, in the foreground, the surface of the adjacent fjord is completely obscured by the very large numbers of icebergs resulting from the recent breakup of the floating glacier tongue and the continuing flux of ice to the margin. The icebergs themselves have similarly crevassed surfaces before fragmentation and overturn.

Satellite and airborne radar and laser altimeter data sets complete the picture of a changing Greenland Ice Sheet. Above the 2000-m contour, representing 70% of the ice-sheet surface, elevations increased by a mean of 5 to 6 cm $year^{-1}$ (*11*, *12*). These values were based on satellite radar altimeter data acquired between 1992 and 2003 (*11*, *12*), and are greater than those reported previously from more scattered airborne evidence (*13*). The pattern of change was variable, however, with growth of 10 to 20 cm $year^{-1}$ in southwest and parts of east Greenland and negative values of 25 to 30 cm $year^{-1}$ in some lower-elevation western areas in particular (*11*, *12*). By contrast, peripheral thinning of the ice sheet was recorded, exceeding 1 m $year^{-1}$ close to the coast, often associated with outlet glaciers (*13*). The thinning was due to changes in ice flow, in addition to enhanced melting. However, parts of southern Greenland appear to be thickening even close to the ice margin, perhaps resulting from increased coastal precipitation (*12*).

Taking the new evidence on the acceleration of ice-sheet outlet glaciers together with estimates of increasingly negative surface mass balance (*7*) yields, according to Rignot and Kanagaratnam (*2*), a contribution from the Greenland Ice Sheet of more than 0.5 mm $year^{-1}$ to global sea-level rise, over two-thirds of which is derived from flow acceleration. This new information on velocity change more than doubles previous estimates of losses from the ice sheet to the global ocean (*6*, *7*). Future monitoring of the velocity structure of the ice sheet, especially above 70°N where acceleration to date has been limited, is required. It is also necessary to understand better the nature and distribution of precipitation over Greenland. Increased accumulation in the ice-sheet interior, and even in some coastal areas, could offset losses attributable to surface melting at lower elevations (*12*). Existing and forthcoming satellites will continue to measure ice-surface elevation and any shifts in the rates of surface melting and accumulation. In a warming world, it is likely that the contribution to sea-level rise from Greenland is set to grow further, assuming that the observed acceleration in outlet-glacier velocities is sustained, with possible increases in precipitation providing the only prospect of short-term amelioration.

Icebergs. Large numbers of bergs are calved each year from the fast-flowing terminus of Kangerdlugssuaq Glacier, East Greenland, adding fresh water to the surrounding seas when they melt.

PHOTO CREDITS: J. A. DOWDESWELL

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Reproductive Social Behavior: Cooperative Games to Replace Sexual Selection

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Theories about sexual selection can be traced back to Darwin in 1871. He proposed that males fertilize as many females as possible with inexpensive sperm, whereas females, with a limited supply of large eggs, select the genetically highest quality males to endow their offspring with superior capabilities. Since its proposal, problems with this narrative have continued to accumulate, and it is our view that sexual selection theory needs to be replaced. We suggest an approach that relies on the exchange of direct ecological benefits among cooperating animals without reference to genetic benefits. This approach can be expressed mathematically in a branch of game theory that pertains to bargaining and side payments.

A recent review of diversity in animal reproductive social behavior (*1*) raises questions about Darwin's 1871 theory of sexual selection (*2*). Unlike the theories of evolution through common descent and of evolutionary change by natural selection, Darwin's theory of sexual selection has continually drawn criticism from evolutionists, notably Huxley in 1938 (*3*). Darwin wrote "Males of almost all animals have stronger passions than females" and "the female… with the rarest of exceptions is less eager than the male… she is coy." Darwin explained these templates as resulting from females choosing mates who are "vigorous and well-armed… just as man can improve the breed of his game-cocks by the selection of those birds which are victorious in the cock-pit." He continues, "Many female progenitors of the peacock must… have… by the continued preference of the most beautiful males, rendered the peacock the most splendid of living birds."

Since 1871, sexual selection theory has often been restated (*4*), yet contemporary definitions share Darwin's central narrative: "We now understand… Males, who can produce many offspring with only minimal investment, spread their genes most effectively by mating promiscuously… Female reproductive output is far more constrained by the metabolic costs of producing eggs or offspring, and thus a female's interests are served more by mate quality than by mate quantity" (*5*). This narrative is taught in biology textbooks (*6*), is axiomatic to evolutionary psychology (*7*), and is broadcast in popular media (*8*).

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The reproductive social behavior of most species has not been studied, but a great many of those that have been do not conform to Darwinian sexual-selection templates. We suggest that sexual selection is always mistaken, even where gender roles superficially match the Darwinian templates.

There are fundamental problems that universally undercut all applications of sexual selection theory to any species, including the contradiction between sexual selection's rationale and the reason for sexual versus asexual reproduction, the difficulty of sustaining a stable hierarchy of genetic quality within a gene pool in the face of continued directional selection for high-ranked genotypes, and the use of different fitness definitions for males and females. These and other fatal problems are detailed in the references accompanying table S1.

We think that the notion of females choosing the genetically best males is mistaken. Studies repeatedly show that females exert choice to increase number, not genetic quality, of offspring and not to express an arbitrary feminine aesthetic. Instead, we suggest that animals cooperate to rear the largest number of offspring possible, because offspring are investments held in common. We therefore propose replacing sexual selection theory with an approach to explaining reproductive social behavior that has its basis in cooperative game theory. We introduce a notion of allocating time into various relationships to maximize cooperative, or "team," fitness. In this theory, we can observe that diverse social organizations emerge from how individuals accrue direct benefits from the relationships they develop with one another within diverse ecological contexts.

Cooperative Games in Reproductive Social Behavior

Here, we explain reproductive social behavior in developmental time, not evolutionary time. A social system develops from the interaction of individuals just as body parts develop from the interaction of tissues. In our model, each animal acts continually as an individual or as a team member, and the value of an action is scored by how it contributes to that animal's average fitness accumulation rate (*9*). An individual's actions involve obtaining and exchanging direct benefits to increase the number of offspring successfully reared (*10*–*14*). We further envision a future two-tier theory that will embed this phenotypic treatment within an overarching evolutionary-genetic model.

Maynard Smith introduced game theory to biology in the 1980s, including the evolutionary stable strategy (ESS), a population-genetic counterpart to the Nash competitive equilibrium (NCE) of game theory (*15*). A competitive game ends when an NCE is attained, i.e., the state where each player cannot better its position, given the positions of the other players. In competitive games, the players do not communicate.

In cooperative games, players make threats, promises, and side payments to each other; play together as teams; and form and dissolve coalitions. Cooperative games usually end up at different solutions to an NCE. Nash also investigated cooperative games and introduced the concept of a Nash bargaining solution (NBS) as an outcome of these games (*16*).

Logic of bargaining and side payments. To illustrate, consider a "payoff matrix" that indicates the direct benefit each player receives in every scenario (*17*):

		Player 2	
		A	B
Player 1	A	(2, 6)	(10, 5)
	B	(4, 8)	(0, 0)

In the upper left, for example, (2, 6) represents a benefit of 2 to player 1 and 6 to player 2 whenever player 1 does action A at the same time as player 2 also does action A. The symbols A and B may have different meanings for each player, because they may be different types of individual: one female and the other male, or one with territory and the other without. Individual competitive play in this example winds up at the lower left, an NCE, because player 2 always moves into the left column ($6 > 5$ and $8 > 0$), and, once in the left column, player 1 always moves down ($4 > 2$). This outcome does not result in cooperative play.

Once this NCE has been attained, player 1 observes it could attain a benefit of 10 if player 2 could be induced to play B when it plays A. Player 1 may therefore "threaten" player 2 by promising to play A, which will reduce player 2's benefit from 8 to 6. For this threat to be

credible, player 1 must demonstrate a willingness to suffer a loss itself from 4 to 2. In response to a credible threat, player 2 can negotiate to play B sometimes when player 1 is playing A, in return for player 1 not carrying out the threat too often. Conversely, player 2 can also counter-threaten player 1. These threats establish a "threat point," i.e., the greatest benefit each can receive is when the other is playing to hurt its opponent the most. Nash suggested that the rational outcome of negotiation is the set of "coordinated" plays by both players acting together as a team that maximizes the "product" of the average benefits each player receives relative to its threat points. We call this product of individual fitnesses the team fitness function. Its maximum is the NBS.

Continuing from the NBS, player 1 may promise a side payment to player 2 to induce it to play B when player 1 plays A. If player 1 offers 4 to player 2, then the upper right entry of the payoff matrix becomes (6, 9). In this new game, the upper right is more profitable than the lower left to both players. The lower left remains an NCE, however, and the upper right becomes an NCE too. If the players find themselves in the lower left, they may agree to play jointly as a team, with player 1 playing A and player 2 playing B, which will then move them to the upper right. Assuming player 1 continues to honor the promised side payment, the upper right is stable even if the players sometimes dissolve into individual play, because the upper right is both an NCE and an NBS.

How might we use this logic to explain social behavior as a cooperative process? The economic and game-theoretic literature presents many models of bargaining tactics to attain an NBS (*18*–*23*). Here, we offer an approach tailored to how reproductive social behavior may develop.

State variables for social dynamics. We suppose each player has a time budget and can allocate a fraction of time into playing each strategy. We assume the animals continually adjust their time allocations into various social relationships to increase their average fitness accumulation rates. Suppose the animals are playing as individuals. If an animal is spending, say, 50% of its time doing A, which is more profitable than doing B, then in the next hour we assume it will increase the time spent doing A and reduce the time spent doing B. Meanwhile, the other animal is also adjusting its time allocations into its A and B actions. A social system then emerges from the simultaneous mutual adjusting of each player's time allocations into the actions most profitable to themselves. When the players play independently, the two state variables are $p_1(t)$ and $p_2(t)$, which are the fractions of seconds during hour t that player 1 and player 2 each play their strategy A.

Alternatively, the animals may play jointly as a team. If the team is spending, say, 50% of its time jointly playing AA, which is more profitable than playing AB, BA, or BB, the team will increase its fraction of time playing AA and reduce the time playing the other combinations. A mixed team strategy is quite possible too, in which the team plays a pair of combinations, say AB and BA, at some best ratio of time. Team play requires four state variables: $x_{AA}(t)$, $x_{AB}(t)$, $x_{BA}(t)$, and $x_{BB}(t)$ are the fraction of seconds during hour t that player 1 and player 2 jointly play each possible pair of strategies. When the animals form a team after playing as individuals or when the team dissolves into individual play after playing as a team, the dynamics for their time allocations switch from the equations that describe individual play to those that describe team play and vice versa, a problem in the optimal control of hybrid dynamical systems (*24*–*27*).

The direct benefits each receives from their interactions with the others are summarized in the payoff matrix whose entries are $w_{ij,k}$, which is the fitness accumulation rate obtained by player k when player 1 plays strategy i and player 2 plays strategy j.

Individual play dynamics. If the individuals independently adjust their time allocations to increase their own average fitness accumulation rates, then the time allocation dynamics consist of two coupled equations. Each individual adjusts its time allocation to climb the gradient of its own average fitness accumulation rate (*28*).

$$\frac{dp_i}{dt} = \frac{1}{w_i(p_1, p_2)}\left(\frac{\partial w_i(p_1, p_2)}{\partial p_i}\right)p_i(1 - p_i)$$

where $i \in \{1,2\}$ and the individual average fitness accumulation rates are $w_i(p_1, p_2) = p_1p_2w_{AA,i} + p_1(1-p_2)w_{AB,i} + (1-p_1)p_2w_{BA,i} + (1-p_1)(1-p_2)w_{BB,i}$.

Team play dynamics. In team play, the team adjusts its time allocation into the four possible combination plays by climbing the gradient of the team fitness accumulation rate (*29*).

$$\frac{dx_{ij}}{dt} = \frac{1}{w_1(x_{AA}, x_{AB}, x_{BA}, x_{BB})w_2(x_{AA}, x_{AB}, x_{BA}, x_{BB})} \times \left\{\frac{\partial^*[w_1(x_{AA}, x_{AB}, x_{BA}, x_{BB})w_2(x_{AA}, x_{AB}, x_{BA}, x_{BB})]}{\partial^* x_{ij}}\right\} \times x_{ij}(1 - x_{ij})$$

where $ij \in \{AA, AB, BA, BB\}$ and ∂^* is a directional partial derivative indicating that, as some variable is changed, the remaining variables alter in unison as a block in the opposite direction, preserving the sum of the $x_{ij}(t)$ equal to one. To form the team fitness accumulation rate, let v_i be the threat point to player i, i.e., player i's best fitness accumulation rate when the other player is playing to minimize it. The individual average fitness accumulation rates in light of the threat point are $w_1(x_{AA}, x_{AB}, x_{BA}, x_{BB}) = x_{AA}(w_{AA,i} - v_i) + x_{AB}(w_{AB,i} - v_i) + x_{BA}(w_{BA,i} - v_i) + x_{BB}(w_{BB,i} - v_i)$ where $i \in \{1, 2\}$. The team fitness accumulation rate is then the product of these individual fitness accumulation rates.

Hence, in the scenario where individual play leads to the competitive solution (NCE) consisting of player 1 playing B and player 2 playing A, a payoff of 4 is yielded to player 1 and 8 to player 2 (Fig. 1). In contrast, team play leads to the bargaining solution (NBS), which consists of playing the combination AB about a quarter of the time and BA about three-quarters of the time, yielding a payoff of about 6 to player 1 and 7 to player 2.

Biological approaches to cooperation have relied on altruism theory and usually wrestle with how to prevent cheaters and free riders (*30*). The bargaining approach to cooperation does not suffer from difficulties with cheating. Should an animal decide not to cooperate, then the other party reverts to the threat point, which is worse for the noncooperator than the bargaining solution. Instead, the difficult issue for the cooperative game approach is whether the animals can carry out team play and can discern the team fitness function whose gradient they should climb. Many animal behaviors involving physical intimacy, such as grooming, traveling, and sleeping in close proximity, making reciprocal interlocking vocalizations, and same-sex and between-sex sexuality could all promote coordinated action. Further, we hypothesize that a sense of friendship resides in animal bonding, a joy or synergy in the spirit of cooperation that allows animals to sense and experience the product, not merely the sum, of their individual well-beings.

Economic approaches to cooperation have focused on repeated games. These encompass what are known as folk theorems, in which many kinds of equilibria are possible in infinitely repeated games, including mutually beneficial ones labeled "cooperative" (*30*–*32*). The classic example is the tit-for-tat strategy in the repeated prisoner's dilemma game (*33*). In repeated games, one party can punish another. When players discern that certain moves lead to punishments, the individuals can wind up moving to a mutually beneficial equilibrium, creating the illusion of cooperation. Our dynamics constitute a repeated game too, because we assume the animals are playing continuously as they adjust their time allocations to actions A and B, if they are playing as individuals, or to AA, AB, BA, and BB, if they are playing as a team. The main difference between previous approaches and ours is how the game is played: Through reciprocal calls and physical intimacy, players perceive team fitness and act accordingly rather than play solely as individuals. Communication during courtship permits bargaining and promises of side payments. The distinction between our proposition and previous work is apparent in the use of the word "cooperative,"

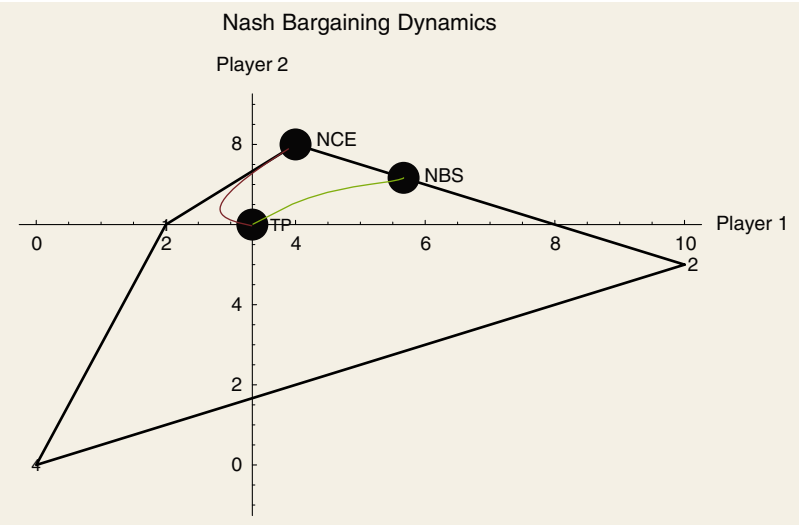

Fig. 1. Comparison of social system resulting from individual play versus team play. The corners of the polygon represent the fitness payoffs at the pure strategies (each player individually chooses to play one strategy 100% of the time). The origin is placed at the threat point (TP) of ($10/3$, 6) to player 1 and player 2, respectively. The trajectory of payoffs each player receives as the social system develops is depicted in brown for individual play and green for team play. Starting at the threat point, individual play culminates at the competitive-equilibrium social system (NCE) where player 1 always plays B and player 2 always plays A, yielding payoffs of 4 and 8, respectively. Starting from the same point, team play culminates at the bargaining-solution social system (NBS) where player 1 and player 2 jointly play AB $5/18$ of the time and jointly play BA $13/18$ of the time, yielding payoffs to player 1 and player 2 of 5.7 and 7.2, respectively.

which means only a mutually beneficial outcome in previous work but describes a process of perceiving and playing the game in our work.

Peacock wrasse game. Peacock wrasses (*Symphodus tinca*) live in shallow rocky habitat off the Corsican coast (*34*, *35*). Females choose whether to lay eggs in a male's nest or to broadcast their eggs over the sea floor. Males defending eggs against predators lose weight and suffer higher mortality. They abandon nests that haven't accumulated many eggs. Abandoned eggs quickly attract predators. The following game may account for when a cooperative male-female association forms versus when male and female pursue their reproductive objectives independently:

		Male	
		Search	Stay
Female	Broadcast	(1, 1)	(0, 0)
	Deposit	(0, 0)	(2, 2)

The female can lay eggs in a territory and the male can remain there to guard them, forming a cooperative couple with both receiving the maximum fitness of 2. Alternatively, they may both remain single, with the female broadcasting her eggs on the sea floor and the male independently searching for them. If so, each receives a fitness of 1, reflecting the loss of some exposed eggs and the failure of the male to find some of them. Or, the female could lay eggs in a territory that the male decides to abandon, resulting in a fitness of 0 for both. Conversely, the male could set up a territory that the female declines to lay eggs in, again resulting in a fitness of 0 for both because the female's eggs spread over the ocean floor are not fertilized. Given this scheme of payoffs, what social system develops?

Under individual play, broadcast-search and deposit-stay are both locally stable Nash competitive equilibria. If we assume the noncooperative solution of broadcast-search is primitive, yielding a fitness of 1 to each, how can the peacock wrasse develop the cooperative solution of deposit-stay that will yield a fitness of 2 for each? Perhaps they bargain.

The female could deposit eggs in a territory-like space regardless of whether the male is there. She might lose them all, but she has driven the male's fitness to 0. Conversely, the male might defend a territory without eggs. His fitness will be 0, but so will the female's. Indeed, the threat point is ($2/3$, $2/3$); the best the female can do if the male is trying to minimize her fitness and vice versa for the male. Because $2/3$ is less than 1 that each is presently receiving, both need to alter their relationship. With courtship and intimate physical contact, they can synchronize activities and play as a team instead of as individuals. The dynamics of team play quickly converge to the cooperative solution of deposit-stay because this is the state with the highest team fitness. In this way, cooperative reproductive social behavior emerges from enlightened Darwinian self-interest.

Team play is not the only dynamic capable of reaching the deposit-stay equilibrium. This outcome is also the risk-dominant and payoff-dominant equilibrium (*36*, *37*).

Oystercatcher game. In the Eurasian oystercatcher (*Haematopus ostralegus*), a sexually monomorphic wading bird common on mudflats, some reproductive groups consist of threesomes with one male and two females, whereas most consist of one male and one female (*38*–*40*). The threesomes occur in two forms, aggressive and cooperative.

In an aggressive threesome, each female defends her own nest, and the male defends a territory encompassing both females. The females lay eggs about two weeks apart. The females attack each other frequently throughout the day. The male contributes most of his parental care to the first-laid eggs, leaving the second nest often unguarded.

In a cooperative threesome, the two females share one nest; both lay eggs in it together, about one day apart; and all three birds defend it together. The females mate with each other frequently during the day, only slightly less often than they do with the male. The females also sit together and preen their feathers together.

How might cooperation instead of aggression develop in oystercatcher threesomes? Consider a three-player game with two females and one male. The benefit each player receives corresponds to the number of their own young successfully fledged. Each female has the option of befriending or attacking the other female. The male has the choice of providing care for offspring of female 1 or female 2. We list the payoffs and strategies in the order (female 1, female 2, male).

To develop the payoff matrix, first suppose male guards female 1's nest and ignores female 2's nest, unless both females share the same nest. When both females befriend each other, the brood consists of two offspring from each, corresponding to benefits of (2, 2, 4). Next, when female 1 attacks female 2, suppose the benefits are (3, 0, 3), indicating that female 1 benefits from male's undivided attention and raises a brood of three eggs consisting only of her offspring, whereas female 2 loses everything because male is leaving her nest unguarded; meanwhile male sees his total payoff drop to three. Alternatively, if female 2 attacks female 1, then female 1 retains some of her eggs because of male's protection, while female 2 can raise two eggs, resulting in payoffs of (1, 2, 3). The asymmetry, (3, 0, 3) and (1, 2, 3), highlights male's care of only female 1's offspring. Lastly, when the females attack each other, both suffer, resulting in payoffs of (1, 1, 2). This situation, where male

helps female 1, is summarized in the first matrix below, and a similar argument for when male helps female 2 leads to the second matrix below:

Male: help 1		Female 2	
		Befriend	Attack
Female 1	Befriend	(2, 2, 4)	(1, 2, 3)
	Attack	(3, 0, 3)	(1, 1, 2)

Male: help 2		Female 2	
		Befriend	Attack
Female 1	Befriend	(2, 2, 4)	(0, 3, 3)
	Attack	(2, 1, 3)	(1, 1, 2)

Four Nash competitive equilibria correspond to both females attacking each other, (attack, attack, help 1) and (attack, attack, help 2), and to the female without the male's support attacking the female who does have his support, (befriend, attack, help 1) and (attack, befriend, help 2).

The threat point for each player is found by assuming the other two players are playing jointly against them. For example, female 2's threat point is found from the following two-player game, in which a coalition's payoff is the sum of the payoffs to its members:

	Coalition of female 1 and male			
	Befriend, help 1	Befriend, help 2	Attack, help 1	Attack, help 2
Befriend	(2, 2, 4)	(2, 2, 4)	(3, 0, 3)	(2, 1, 3)
Female 2				
Attack	(1, 2, 3)	(0, 3, 3)	(1, 1, 2)	(1, 1, 2)

The female 1–male coalition can play four strategies, and female 2, two strategies. If this coalition plays to minimize female 2, it will move to the third column, (attack, help 1), and then the best female 2 can do is move to the bottom row, (attack), yielding her a payoff of 1. This is threat point against female 2, the best she can do in the worst case. This logic yields the following threat points for all individual players:

Coalition structure	Threat point
{Female 1, male} versus {female 2}	1
{Female 2, male} versus {female 1}	1
{Female 1, female 2} versus {male}	2

In a fully cooperative game, the three players choose their strategies jointly. The NBS maximizes the product of the three players' benefits. The NBS is to play (befriend, befriend, help 1) 50% of the time and (befriend, befriend, help 2) the remaining time. This solution is attained through team-play dynamics and represents the two females caring for their offspring jointly and the male splitting his efforts equally between the offspring of female 1 and female 2, as automatically occurs when both females share the same nest.

Conclusion

Cooperative game theory is the mathematical basis for social selection, an alternative to sexual selection theory (*1*). The key elements to social selection are: (i) Reproductive social behavior and sexual reproduction are cooperative. Sexual conflict derives from negotiation breakdown. In sexual selection, sexual conflict is primitive and cooperation derived, whereas in social selection sexual cooperation is primitive and conflict derived. Hence, sexual selection and social selection are mutually exclusive theories. (ii) Within reproductive social groups, organisms bargain and trade direct ecological benefits to maximize number of young reared. (iii) Reproductive groups are coalitions of one or both sexes that may include prezygotic ''helpers,'' such as the white-collared male ruff and feminine male bluegill sunfish who assist in courtship, together with postzygotic helpers who assist in raising offspring. Families are reproductive groups whose participants share kinship. (iv) Secondary sex characters are social-inclusionary (SI) traits that permit participating in the species' social system, and exclusion is reproductively lethal. Two types of SI traits include (i) cooperation facilitators like mutual grooming and preening, interlocking vocalizations, between-sex and same-sex sexuality, and other intimacies promoting coordinated team play and the perception of team fitness and (ii) expensive, functionally useless badges like the peacock's tail that are admission tickets to monopolistic resource-controlling coalitions. Any imperfection in an admission ticket is the target of prejudice. In social selection theory, cooperation and team play coexist with prejudice and exclusion.

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6. "Male birds are often beautiful; and the female of the species is a drab, camouflaged creature… [because] female birds just want to mate with males decked out in finery… Why have colorful appearances evolved so rarely [in animals other than birds]?… [because] flight may allow bird species to indulge the taste for gaudiness that many females apparently have (*41*)."
7. "Because women in our evolutionary past risked enormous investment as a consequence of having sex, evolution favored women who were highly selective about their mates… A man in human evolutionary history could walk away from a casual coupling having lost only a few hours of time (*42*)."
8. "Males fighting for females is the elastic in the jockstrap of evolution, therefore women are hardwired to 'size up' and appreciate male competition (*43*)."
9. An individual's rate of fitness accumulation is the integrand in the demographic formula for an animal's lifetime reproductive success, $R_o = \int_0^\infty m(x)l(x)\,dx$. $m(x)$ is fecundity per time at age x, and $l(x)$ is the probability of living to age x or more; their product is a fitness accumulation rate. In developmental time, animals choose actions to increase their instantaneous $m(x)l(x)$. Lifetime fitness, R_o, needed in evolutionary models, is the fitness accumulated during the expected lifespan.
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28. These equations resemble coevolution between two species, each with one haploid locus containing two alleles. Here, the notion of a "seconds pool" replaces that of a gene pool, profitable seconds beget more profitable seconds with haploid inheritance, and the time-allocation variables change as each individual climbs their own adaptive surface, by analogy to the adaptive topography metaphor of population genetics.
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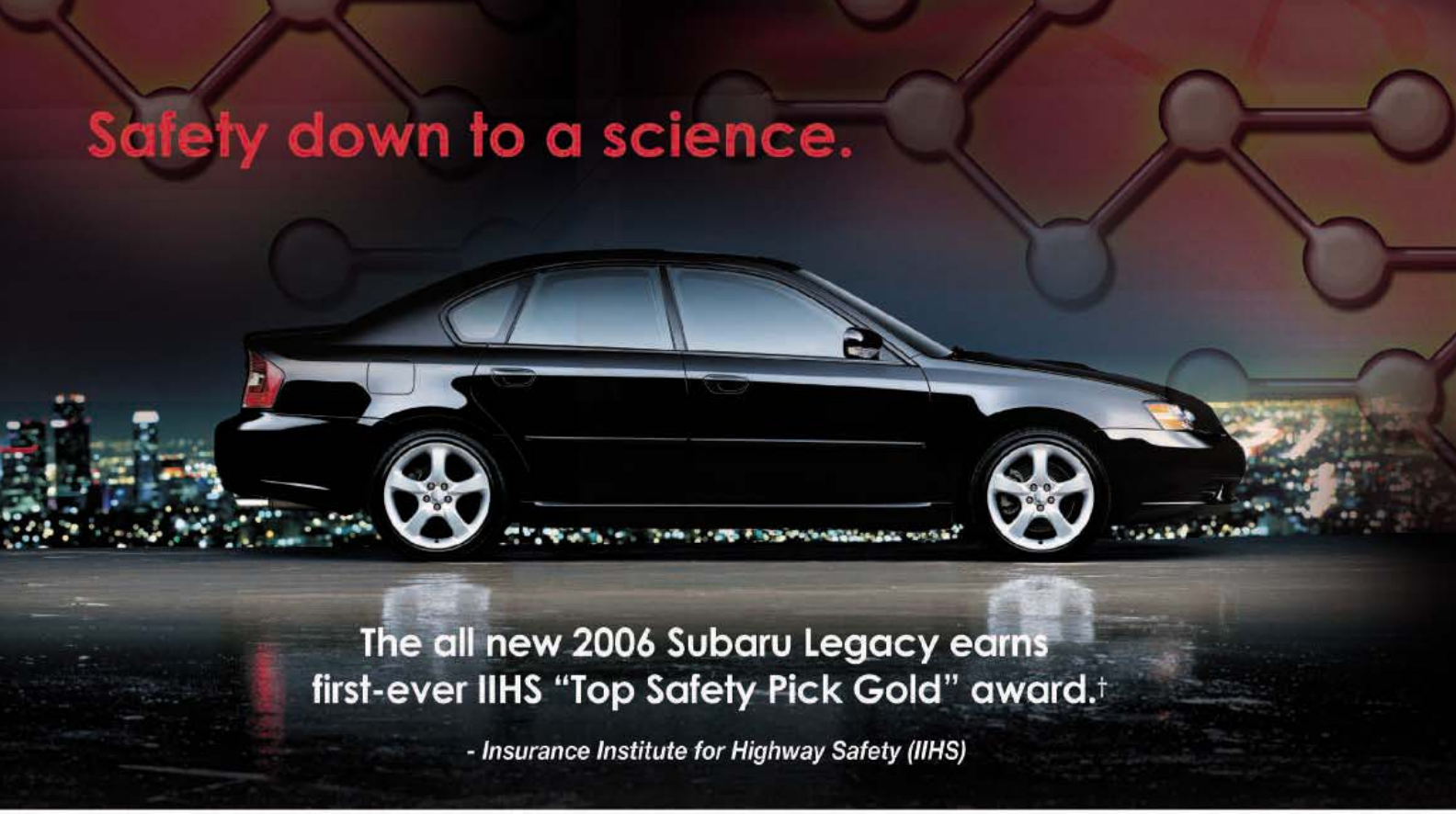
Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5763/965/DC1
Table S1
References and Notes

10.1126/science.1110105



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Genetic Variation Affects de Novo Translocation Frequency

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Translocation is one of the most frequently occurring human chromosomal aberrations. Balanced carriers usually manifest no phenotype but experience problems with reproduction. These include infertility, recurrent abortion, and offspring with chromosomal imbalance. The constitutional t(11;22)(q23;q11) is a balanced translocation between chromosomes 11 and 22, with breakpoints at bands 11q23 and 22q11. It is the only known recurrent non-Robertsonian translocation and represents a good model for studying translocations in humans (*1*). The recurrent nature of this translocation prompted us to examine t(11;22) breakpoints for a specific genomic structure. The analysis of many unrelated t(11;22) cases revealed that the breakpoints occur within palindromic AT-rich repeats (PATRRs) on 11q23 and 22q11 (PATRR11 and PATRR22) (*2*–*4*). The majority of the breakpoints are localized at the center of the PATRRs, suggesting that the center of the palindrome is susceptible to double-strand breaks (DSBs), thereby inducing illegitimate chromosomal rearrangement (*3*, *5*). Recent findings of PATRR-like sequences at the breakpoints of other translocations support the possibility that palindrome-mediated chromosomal translocation is a general pathway for human genomic rearrangements (*6*–*8*).

The PATRR11 is variable in size in normal healthy individuals (Fig. 1 and table S1). The most common allele is a ~450–base pair (bp) PATRR11 (L-PATRR11) that forms a nearly perfect palindrome (*5*). Several types of short variants were identified (S-PATRR11) that appear to be derived from the longer version primarily by deletion near the symmetric center of the palindromic structure. We can classify the S-PATRR11s into four groups. The most frequent 350-bp variant, S1-PATRR11, has a 50-bp deletion at both of the palindromic arms but still remains completely symmetrical. S2-PATRR11 has an asymmetric deletion at its center, but the new center manifests a symmetric palindrome. S3-PATRR11 does not possess palindromic features by virtue of a deletion at the center of the palindrome. We identified a rare 434-bp S-PATRR11, which sustained an asymmetric central deletion followed by the insertion of an AT-rich sequence of unknown origin (S4-PATRR11). We also identified another rare allele with a duplication of the proximal arm, which constitutes a 603-bp asymmetric palindrome (EL-PATRR11). On the basis of the palindrome-mediated mechanism of the translocation, it is reasonable to hypothesize that the polymorphism of the PATRR11 could affect the frequency of de novo t(11;22) translocations.

Translocation-specific polymerase chain reaction (PCR) detects de novo t(11;22) translocations in normal sperm samples from healthy individuals with a normal karyotype (*9*). We analyzed sperm DNA from individuals with various genotypes for the PATRR11. Five men homozygous for the L-PATRR11 genotype produced de novo translocations at a frequency ranging between 1.52×10^{-5} and 1.57×10^{-4} (table S2). A heterozygote for the L-PATRR11 and S1- or S2-PATRR11 alleles produced de novo translocations at a similar overall frequency, as did homozygotes for the L-PATRR11. Sequence analysis of the PCR products revealed that products derived from the symmetrical S1- or S2-PATRR were less frequently observed than products originating from the L-PATRR11, with a low estimated frequency of de novo translocations ($\sim 10^{-6}$), suggesting that the frequency of de novo translocation depends on the size of the PATRR. On the other hand, although two individuals heterozygous for L-PATRR11 and S3-PATRR11 produced de novo translocations at a similar overall frequency, sequence analysis revealed that virtually all of the de novo translocations appeared to originate from the L-PATRR11. This finding suggests that the asymmetric S-PATRR does not produce de novo translocations (table S2).

Sperm from one individual, who was a rare compound heterozygote for the S1- and S3-PATRR11, contained de novo translocations at a very low frequency. Sequence analysis of the junction fragments showed that the translocations were exclusively derived from the S1-PATRR11. Because both the S2- and S4-PATRR11s alleles produced translocations at a low frequency ($\sim 10^{-6}$ and $\sim 10^{-7}$, respectively), a compound heterozygote for this genotype had a very low translocation frequency overall. We identified another individual heterozygous for the S3-PATRR and EL-PATRR. This individual is the only person who carried asymmetric PATRR11s on both alleles. He did not produce any de novo translocations (table S2). The frequencies for each allele are summarized in Fig. 1.

This work demonstrates genetic variation over more than three orders of magnitude in the susceptibility for generating the recurrent translocation in humans. Our results point to the importance of genomic sequence variation on the frequency of chromosomal rearrangements, a class of human mutation that is generally thought to be random. Our data imply that genetic variation plays an important role in this process.

Allele type	Subtype	Size (bp)	Number	Allele frequency (%)	Structure	Translocation frequency
L-PATRR11		442-450	343	87.1		$1.07\text{-}2.27 \times 10^{-5}$
S-PATRR11	Type 1	350	20	(40.0)		$1.20\text{-}2.93 \times 10^{-6}$
	Type 2	292-410	5	(10.0)		$1.55\text{-}1.71 \times 10^{-6}$
	Type 3	63-380	24	(48.0)		$<3.05 \times 10^{-7}$
	Type 4	434	1	(2.0)		6.81×10^{-7}
	Subtotal		50	12.7		
EL-PATRR11		603	1	0.3		$<1.69 \times 10^{-7}$
Total			394	100.0		

Fig. 1. Polymorphic PATRR11 alleles and their translocation frequencies. Arrows indicate each unit of inverted repeats. Vertical arrowheads indicate the center of the palindrome. Dotted blue lines show deleted regions, whereas red lines indicate insertions. Calculated frequencies of de novo translocation produced from each allele are shown at the right.

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Supporting Online Material

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Materials and Methods
Tables S1 to S3

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The Second Ring-Moon System of Uranus: Discovery and Dynamics

Mark R. Showalter[1]* and Jack J. Lissauer[2]

Deep exposures of Uranus taken with the Hubble Space Telescope reveal two small moons and two faint rings. All of them orbit outside of Uranus's previously known (main) ring system but are interior to the large, classical moons. The outer new moon, U XXVI Mab, orbits at roughly twice the radius of the main rings and shares its orbit with a dust ring. The second moon, U XXVII Cupid, orbits just interior to the satellite Belinda. A second ring falls between the orbits of Portia and Rosalind, in a region with no known source bodies. Collectively, these constitute a densely packed, rapidly varying, and possibly unstable dynamical system.

The Voyager 2 spacecraft revealed the planet Uranus to be accompanied by a family of small, inner moons that inhabit the region between the ring system and the larger classical satellites. Using the Hubble Space Telescope (HST), we have discovered two additional moons and two dusty rings in this region. These findings yield a picture of a "second ring system" of Uranus, comprising 11 dynamically interacting observed moons, one (thus far unseen) tiny or disrupted moon, and the belts of dust that emerge from them.

Images taken by Voyager 2 as it approached its January 1986 encounter with Uranus initially revealed 10 inner moons: two "shepherding" the outermost known ring (ε) and eight others between the ring region and the planet's classical moons (*1*). One additional moon, U XXV Perdita (S/1986 U 10), was later found in a reanalysis of the Voyager images (*2*). The largest of these inner moons, Puck, is relatively isolated at an orbit of 86,004 km. Eight remaining moons, collectively known as the "Portia group" after their largest member, span a rather narrow radial zone, with the innermost (Bianca) at 59,166 km and the outermost, Perdita, at 76,417 km. Orbital simulations of this densely packed region reveal that this family of satellites is chaotic and dynamically unstable over time scales of only 10^6 to 10^8 years (*3*).

Observations and discoveries. We examined the uranian system from July 2003 through August 2005 with the use of the High Resolution Channel (HRC) of HST's Advanced Camera for Surveys (ACS). The HRC has a pixel scale of 0.025 arc sec (350 km at Uranus's opposition distance) and an effective point spread function several times this size. We obtained a large number of long-exposure, clear-filter images to search for tiny moons and faint rings (table S1). With a typical exposure time of 250 s, the planet is saturated by a factor of ~50 in these images. However, the saturated pixels "bloom" along each image's vertical axis, whereas we orient the ring-moon system horizontally (Fig. 1A). The clear filter has an effective bandpass of 0.3 to 0.7 μm (after scaling by the solar spectrum), providing more than twice the throughput of the HRC's next broadest filter, F606W. As a result, our images are substantially more sensitive than the Voyager images.

Our key observations are from four extended "visits" near Uranus's opposition in 2003, 2004, and 2005 (table S1), dedicated to the search for tiny moons. Our first extended visit in August 2003 lasted 4 hours; two visits in August 2004 lasted 7 hours each and were separated by 6 days; and a fourth visit in August 2005 lasted 4 hours. Durations were chosen so that most of the inner uranian moons would execute half an orbit during one visit, ensuring that each moon would be imaged at least once near maximum elongation. Our images are most sensitive to moons at these locations because noise is reduced at greater projected distances from the planet, and because motion smear is minimized. We also obtained two or three long exposures during nine additional brief visits that focused on photometry of the main rings.

Details of our image processing are discussed in (*4*). Our techniques allow for the removal of the strong brightness gradient around the planet and also mask out the prevalent cosmic ray hits that litter long HST exposures. After this processing, the moons U XXV Perdita (S/1986 U 10) (*5*) and U XXVII Cupid (S/2003 U 2) (*6*) appeared in the first images from our extended 2003 visit (Fig. 1B) (movie S1). The more distant moon, U XXVI Mab (S/2003 U 1), appeared in images taken about an hour later. To obtain still finer sensitivity, we can align and coadd the pixels from larger sets of similar images. Two new rings, R/2003 U 1 and R/2003 U 2 (henceforth R1 and R2, respectively) ap-

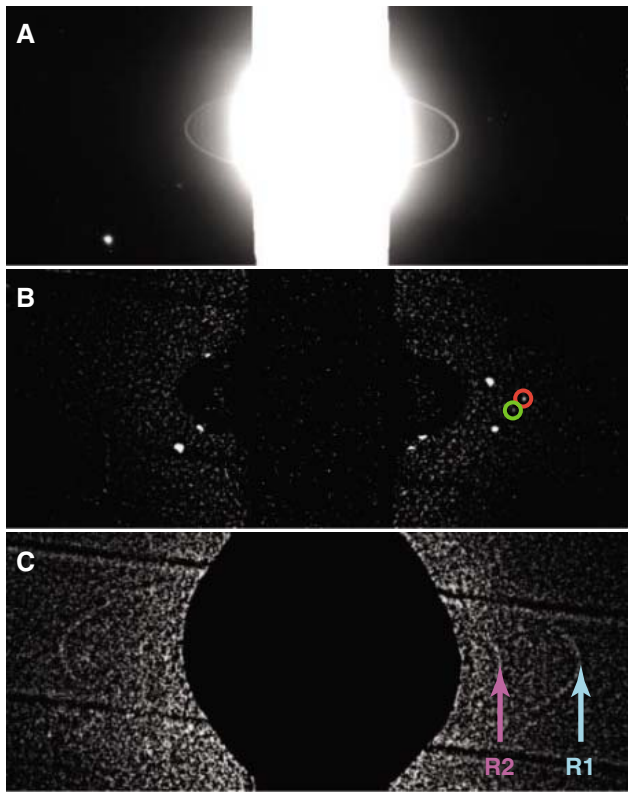

Fig. 1. Three versions of images from August 2003 illustrate our observations, processing steps, and initial discoveries. (**A**) Image J8OA02GXQ is shown with minimal processing to illustrate our observing technique. The planet disappears behind the bright vertical bar because of saturated pixels blooming along this axis. However, the rings and moons are visible to its right and left. (**B**) Our filtering technique eliminates the background gradient and erases bright regions, including most cosmic rays. Contrast enhancement reveals known moons and any point sources fainter than Bianca. Perdita and Cupid are identified by red and green circles, respectively. (**C**) A summation of 24 images from this visit reveals two previously unknown rings, R1 and R2. Note that (B) and (C) have been slightly blurred to reduce noise and improve their appearance in print. The linear features with slight downward slopes from left to right in these panels are the edges of Uranus's diffraction band. The horizontal scale of the image is about 210,000 km.

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peared upon coadding all the images from the 2003 visit (Fig. 1C).

For a more complete picture of the system, moving targets such as moons and arcs must be rotated to a common epoch before coadding. The resulting longitude-radius "maps" show all of the moons and rings in the region (Fig. 2). The two newly discovered moons are identified, as is Perdita, which had not been seen since the 1986 Voyager flyby (*2*). A careful review of these figures does not reveal any convincing evidence of additional moons. Although some maps show suggestive spots, none of these can be reliably tracked from one visit to another. We believe our search is complete down to one magnitude fainter than our faintest moon, Cupid (*4*).

Rings appear as horizontal lines in Fig. 2. Hints of other ring-like structures, perhaps incomplete, can be seen at smaller radii, but the image noise is greater close to the planet, so no additional rings can be confirmed.

Moon photometry and sizes. Because the filtering process adjusts the relative brightnesses of nearby pixels, it renders the images unsuitable for absolute photometry. We instead obtain photometry from the unfiltered images by modeling and subtracting the background gradient surrounding each moon (*4*). Image intensity I is measured by the dimensionless ratio I/F, defined such that πF is the incoming solar flux density. Geometric albedo p equals the disk-integrated I/F divided by a moon's cross-sectional area (*2*). To eliminate variations that might arise from irregular shapes, we integrate I/F only near each moon's maximum elongation, where its cross section is maximized. Because the clear filter is an unsupported feature of the HRC, absolute calibration is poorly defined. However, the relative brightnesses of the Portia group, Puck, and Miranda are a reasonable match for more detailed earlier work (*7*); this defines the calibration that we have used throughout our analysis.

Table S2 lists all of our measurements. Our photometry reveals a previously unknown property of Puck: It is 10% brighter on its trailing side than on its leading side. This may be important because, as we show below, Puck skims the inner edge of R1. None of the other moons we have studied shows evidence for such an asymmetry.

We derived the sizes for the smallest moons by scaling them to the above measurements. Perdita and Cupid fall near the Portia group, where p varies by no more than ~10%. If we assume that Perdita and Cupid have albedos similar to those of their neighbors, then they have radii of 13 and 9 km, respectively (Table 1) (table S2). If Mab has an albedo comparable to that of nearby Puck, then its radius is 12 km, making it slightly smaller than Perdita even though it is 20% brighter. However, Mab orbits between Puck and Miranda, so its surface may more closely resemble that of the latter, much brighter body. If so, then Mab's radius is only 6 km, even smaller than that of Cupid. Without detections that might reveal color relationships between Mab and Puck or Miranda, we can only guess at Mab's actual size.

Astrometry and larger moon orbits. We obtain precise astrometry of all of the moons with the use of standard centroiding techniques (*4*). We fit orbital elements assuming that each moon follows a Keplerian elliptical path modified by the oblateness of the planet. The upper portion of table S3 summarizes the orbital elements for a simultaneous fit of the Portia group, Puck, and Miranda. All measurements are weighted equally, and root mean square (RMS) residuals are <0.3 pixels.

A comparison of these fits with prior work (*8*, *9*) reveals a surprise: Most orbits have changed significantly in the past decade (Fig. 3). Belinda's changes are especially large. Nonetheless, the

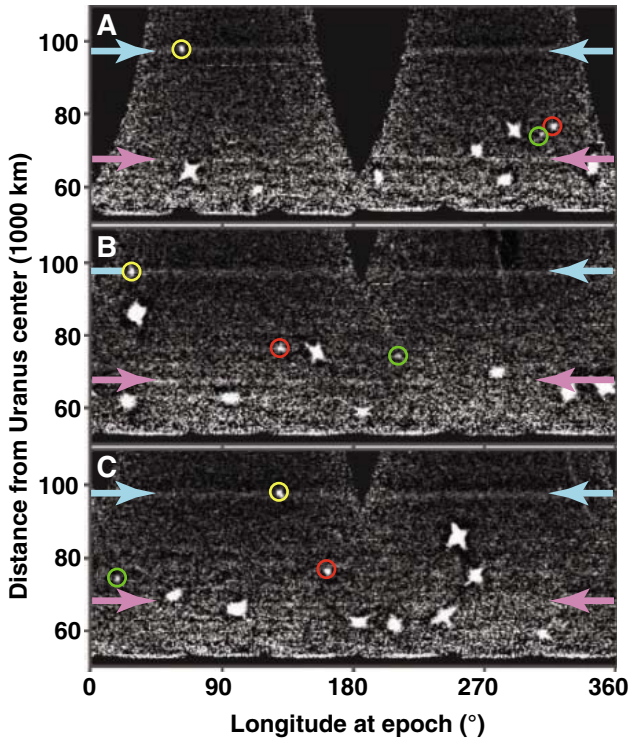

Fig. 2. Maps of our extended visits in 2003 (**A**) and 2004 (**B** and **C**) show all of the known rings and moons. These were generated by combining all available images while rotating features to the midtime of each visit. This summation improves our sensitivity for the smallest moons by overlaying their pixels from multiple images. In this processing, rings appear as horizontal bars. Black wedges appear where data are absent. All 11 moons are visible in (B) and (C); Puck was obscured during the 2003 extended visit (A). Circles identify the smallest moons: Perdita (red), Mab (yellow), and Cupid (green). Horizontal arrows indicate R1 (blue) and R2 (violet). All panels have been slightly blurred to reduce noise and improve their appearance in print.

Table 1. Summary of moon properties. See the text and tables S2 and S3 for details.

Moon	Semimajor axis (km)	Mean motion (°/day)	Eccentricity (10^{-3})	Inclination (°)	Fit residual (pixels)	Integrated I/F (km²)	Mean radius (km)
Bianca	59,165.56	828.38789	0.27	0.181	0.24	149	27
Cressida	61,766.72	776.58279	0.20	0.038	0.20	366	41
Desdemona	62,658.38	760.05540	0.34	0.098	0.26	322	35
Juliet	64,358.23	730.12618	0.05	0.045	0.19	666	53
Portia	66,097.29	701.48633	0.51	0.026	0.18	1065	70
Rosalind	69,926.82	644.63034	0.58	0.093	0.21	295	36
Cupid*	74,392.38	587.44316	——	——	0.67	17	9
Belinda	75,255.61	577.36060	0.28	0.028	0.27	424	45
Perdita*†	76,416.73	564.24640	3.29	0.068	0.58	39	13
Puck	86,004.49	472.54457	0.39	0.321	0.16	2,142	81
Mab*	97,735.91	390.05011	2.54	0.134	0.81	50	12
Miranda	129,848.46	254.69034	0.62	4.449	0.28	81,530	237

*Moons emphasized in this study. †The orbital elements of Perdita assume an additional resonant libration, as discussed in the text and in table S2.

changes in energy and angular momentum are generally centered around zero; a mass-weighted sum is consistent with conservation principles. Note that Portia, by far the most massive moon, has among the smallest changes. Also, Cressida and Desdemona have shifted in opposite directions, consistent with expectations that these moons' orbits are closely coupled (*3*). The members of the Portia group are clearly perturbing one another measurably over brief time scales, supporting predictions that the entire system is chaotic.

Orbit of Perdita. The most notable feature of the initial fits to the remaining three moons is the much larger residuals of 0.65 to 0.9 pixels (table S3). Centroiding these fainter moons is more difficult, but that does not fully explain this result. Extrapolation of Perdita's HST-derived orbit back to 1986 locates it ahead of the Voyager observations by 57° ± 2°, implying that this moon's average speed over the past 18 years has been 0.008°/day faster than it is currently. Perdita clearly does not follow a uniform Keplerian path.

We derive a semimajor axis a = 76,417.45 ± 0.03 km from the HST data, or 76,416.680 ± 0.003 km for the long-term average. These values straddle Belinda's 43:44 outer Lindblad resonance at 76,416.749 ± 0.015 km. To allow for the possibility that this resonance explains the large residuals, we fit an alternative orbit model including libration to the HST and Voyager data (*5*). The residuals drop to 0.6 pixels, a more statistically plausible value that indicates that the 57° offset from the Voyager era has been resolved. The derived full libration amplitude is 7.05° and the period is 3.24 years. Now a = 76,416.731° ± 0.007 km, a precise match for Belinda's resonance. Nonetheless, it should be noted that the libration amplitude approaches the largest possible value of 360°/43 = 8.4°.

The role of this particular resonance had been suggested by Karkoschka (*2*), but at the time it could not be confirmed. This constitutes a definitive identification of a mean motion resonance among Uranus's satellites. Karkoschka also noted that Rosalind's 8:7 outer resonance falls at 76,430.6 km; this near-resonance probably also plays an important role, so a more sophisticated model may describe Perdita's motion more precisely.

Orbit of Mab. Mab also shows very large orbit residuals of 0.8 pixels (Table 1) (table S3). This object is relatively bright in our data and is well isolated from the other moons, which suggests that our measurement errors should not be so large.

The formal uncertainty in the mean motion is 0.00034°/day, amounting to 2.2° in the elapsed 18 years since the Voyager flyby. Because Mab is brighter than Perdita, we have searched for it in the Voyager data set. It appears in four of the most sensitive backscattered images, trailing its extrapolated location by a mere 1.7° (Fig. 4).

To understand the large residuals, we fit subsets of the data, divided by year (Fig. 5). Single-year fits provide residuals of ~0.3 pixels, which probably represent the actual precision of the measurements. However, the orbital elements show marked variations from one year to the next. For example, Mab's orbital position in 2004 fell more than a degree behind that in 2003 and 2005. Other years show smaller but still statistically significant deviations. A large but unknown perturbation is clearly at work on Mab.

Mean motion n varies by 0.12°/day, and λ varies by ~1.5° from the mean orbit (Fig. 5). The time scale T of the libration can be estimated from the amplitude of the deviations: $T \approx 2\Delta\lambda/\Delta n$. This value is 25 days, but it may be a distinct underestimate because the orbit-fitting procedure tries to minimize Δλ. However, no first-order resonances fall in the vicinity of Mab, so the force driving this quite rapid libration is unknown. The changes in mean motion correspond to excursions of 45 km in semimajor axis, a distance too large to allow for a co-orbital satellite less massive than Mab to be responsible. Further observations are needed to understand the peculiar orbit of Mab.

Orbit of Cupid. In comparison to the above, the tiniest moon, Cupid, appears to follow a very simple path (Table 1) (table S2). Its RMS residual of 0.7 pixels is probably reasonable given the intrinsic uncertainties in centroiding such a faint moon. The residuals show no underlying trends to suggest that some component of Cupid's motion has been overlooked. Both the inclination and eccentricity are small relative to their associated uncertainties, so we also fit a circular, equatorial orbit. The increase in residuals is negligible, so we adopt this simple model for the motion of Cupid. Nonetheless, because of fewer detections and larger measurement residuals, Cupid's eccentricity and inclination are not as well constrained as those of the other moons.

The remarkable feature of Cupid is its close proximity to Belinda, orbiting just 863 km interior to it and raising questions about its long-term stability. The only known satellite pairs on closer orbits are in 1:1 resonance. But any interaction with Belinda appears to be nonresonant; Belinda's 59:58 inner Lindblad resonance falls 3 km away, whereas first-order resonances are spaced by just 15 km, so this proximity is statistically insignificant.

Ring profiles. As with the moons, images of the rings are altered in width and amplitude by the high-pass filtering that first revealed their existence (Fig. 1C and Fig. 2). To understand them quantitatively, one must return to the unfiltered images and find alternative methods to handle the background gradient (*4*). The results are radial profiles of each ring, generated by coadding all of the appropriate pixels from all the images of our extended opposition visits (Fig. 6). As expected for optically thin rings, the brightness varies in proportion to 1/(sin B), where B is the ring opening angle (table S1). Thus, we have divided out this factor to yield the "normal I/F," that is, the I/F that would have been observed from a hypothetical perpendicular viewpoint at the same phase (i.e., Sun-ring-observer) angle α (Table 2).

Why did Voyager overlook these rings? The likely reasons are that both rings are extremely faint and that R1 orbits much farther from the

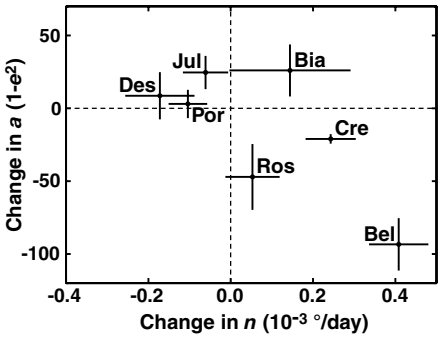

Fig. 3. Changes in orbital elements of each moon in the Portia group since 1994. Current orbital elements (table S3) are compared to earlier work by Jacobson (*9*), who derived precise orbits from the Voyager images of 1986 and HST images from 1994. We plot each moon's change in mean motion n and in the combination $a \times (1 - e^2)$, where a is semimajor axis and e is orbital eccentricity; the former is closely related to orbital energy and the latter to angular momentum. Error bars represent ±1 standard deviation (σ). Several moons, particularly Belinda and Cressida, have shifted by several σ in the new orbital solutions.

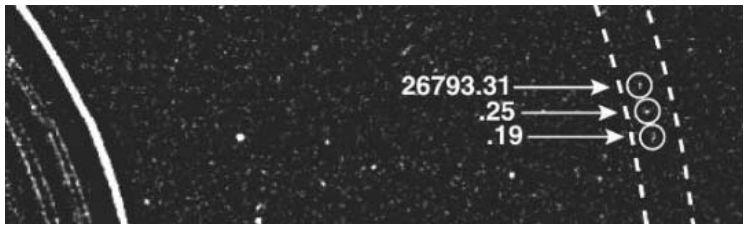

Fig. 4. The recovery of Mab from three Voyager images. The figure was constructed by overlaying strips from images 26793.19, 26793.25, and 26793.31, separated by 4.8 min each. Arrows and circles indicate the location of Mab in each image. For reference, dashed lines mark orbital radii ±2000 km from Mab's nominal orbit. Mab is displaced slightly inward, which suggests that it has been imaged near pericenter. A fourth image not shown, 26794.13, captured Mab again 34 min later.

planet than anyone had expected to find rings. However, as with our HST images, one can substantially increase sensitivity by combining multiple Voyager images. We have examined a sequence of 95 outbound images, 26936.34 to 26973.54, with $\alpha = 146°$ (*4*). After coadding all of the pixels, both rings come into clear view (Fig. 6) and have profiles that almost perfectly match the HST data. A similar analysis of 18 approach (low-phase) images, 26791.25 to 26794.19, did not achieve sufficient sensitivity to detect either ring.

The rings are brighter in absolute terms in the high-phase Voyager data. This clearly indicates that micrometer-sized dust is a major component of each ring. By assuming that macroscopic bodies dominate the rings' backscatter, we can obtain one estimate of the normal optical depth of large particles, τ_{big}: $I/F = p\tau_{big}$. If we assume that p is the same as for the nearby moons, then $\tau_{big} \sim 10^{-5}$ for R1 and R2 (Table 2). These values fall well within the range of other known faint, dusty rings.

The peak of R1 aligns very closely with the orbit of Mab. Other small moons in the solar system are paired with rings, including the four innermost jovian moons, Pan at Saturn and Galatea at Neptune. These are tiny moons from which the dust most likely escapes because of meteoroid impacts or perhaps recollisions from other ring grains (*10*, *11*). The optimal size for ring-producing moons is ~10 km, because larger moons retain most of their ejecta via self-gravity, whereas smaller moons simply have less surface area. Mab falls quite close to the optimal size, which may help to explain its prominent ring. Nonetheless, the other comparable uranian moons Perdita and Cupid do not produce rings that we know of, possibly because Belinda limits the lifetimes of nearby dust. Bianca may be of the appropriate size to produce a ring, but our observations are not sensitive to faint rings so close to the planet.

In contrast to R1, R2 has no known embedded source bodies. Several mechanisms remove or destroy dust grains within planetary rings on short time scales (*10*). Estimates of dust lifetimes in other faint rings range from days to no more than centuries. Thus, a population of unseen parent bodies must be present in the core of this ring. The fact that R2 is relatively more backscattering than R1 supports this inference, because the latter is populated by Mab and probably lacks a population of macroscopic bodies. Saturn's G ring is perhaps the best analog; it also has no visible source bodies, but charged particle absorption data indicate that a population of meter- to kilometer-sized bodies must be present (*12*), which probably maintains the visible dust population in steady state (*13*).

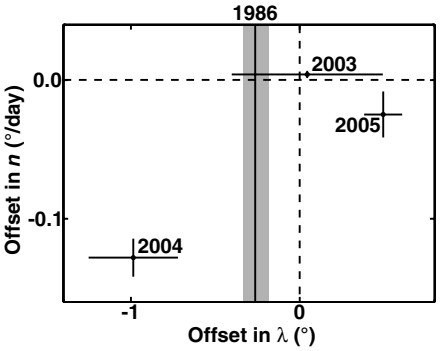

Fig. 5. Year-by-year variations in the orbit of Mab. The origin of the plot represents the best-fit mean longitude λ at epoch and mean motion n for all data from 1986 to 2005. The same quantities based on fits to the data from each individual year are shown for comparison; error bars are ±1σ. For the Voyager data of 1986, uncertainties in n are large, so only the offset in λ and its uncertainty (gray band) are plotted.

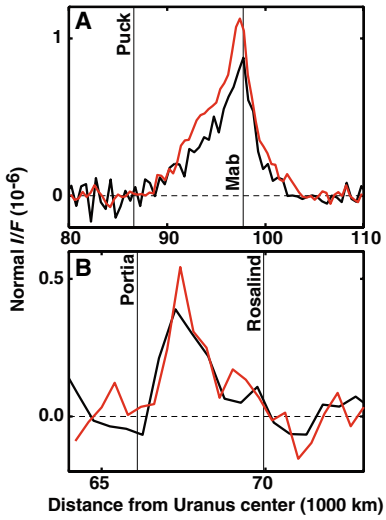

Fig. 6. Radial profiles of the rings R1 (**A**) and R2 (**B**), generated from HST and Voyager images. HST data are shown as black lines; Voyager data are shown in red. Both rings are brighter at Voyager's high phase angle $\alpha = 146°$. To foster comparisons with the HST data, we scaled R1 downward by a factor of 4 and R2 by a factor of 2. The orbits of nearby moons Mab, Puck, Portia, and Rosalind are indicated by vertical lines.

Table 2. Summary of ring properties. See the text and tables S2 and S3 for details.

Ring	Peak radius (km)	Inner limit (km)	Outer limit (km)	HST I/F (10^{-6})	Voyager I/F (10^{-6})	Peak τ (10^{-6})
R1 (R/2003 U 1)	97,700	86,000	103,000	0.88	4.5	8.5
R2 (R/2003 U 2)	67,300	66,100	69,900	0.39	1.1	5.6

Both R1 and R2 share another trait with the G ring: a distinctly triangular profile (*12*, *14*). This may suggest similar dynamics, in which dust ejected from the source bodies achieves a distribution of orbital eccentricities, perhaps because of perturbations by electromagnetic forces or solar radiation pressure. However, it is clear that none of these rings are dominated by drag forces, because such rings would extend primarily inward or outward from their source, depending on the direction of the drag. Jupiter's gossamer rings, bounded by Amalthea and Thebe, are prime examples of drag-dominated rings. Apparently, Uranus's hot, extended exosphere (*15*) does not affect these rings substantially. Asymmetries in the triangular profiles may indicate the direction of any small drag forces that do play a role.

Three of the four ring boundaries coincide with known moons: Portia and Rosalind surround R2, and Puck orbits near the inner edge of R1. These moons could sweep up any material that wanders too far from the source region. The leading/trailing asymmetry of Puck may indicate that ring material preferentially strikes its leading face and darkens it (either by depositing intrinsically darker grains or by uncovering brighter interior material). However, the rings gradually peter out rather than terminating abruptly at the orbits of the moons, so much of the dust within R1 and R2 is probably removed by permanent loss mechanisms. The obvious sinks for the ring dust are the source bodies themselves; because the grains remain in the general vicinity of their origins, the likelihood of reimpacting a source body can be large.

How did the source population for R2 come to exist? It remains possible that a single moon will eventually be found in the region. However, our detection threshold is ~5 km, already rather small for a single moon to produce such a prominent ring. Alternatively, a large number of small but macroscopic bodies may inhabit the region between Portia and Rosalind, giving rise to the telltale dust. These bodies could be the result of a disrupted moon that once orbited at this location. Because R2 falls near the Roche limit, it might never reaccrete; alternatively, the reaccretion is in progress and the ring is therefore transient. The disruption might have been the result of a large external impactor; it is widely believed that other moons of Uranus have been disrupted one or more times in their history (*1*, *16*, *17*). The most tantalizing possibility is that this band of debris is the direct result of the short-term instability of the inner uranian moons. With stability time scales of 10^6 to 10^8 years (*3*), it stands to reason that one or more lost satellites would still have remnants within the system, provided that some collisions between small moons are disruptive rather than accretional.

We note that R2 shows some evidence for variations with longitude. In Fig. 1C, it is measurably brighter on the right ansa. In Fig. 2, R2 is visible in all three panels, but not at all

longitudes; in panels B and C, obtained just 6 days apart, the ring appears to be consistently brightest at longitudes leading (to the right of) Portia, which can be identified in the figure as R2's bright interior neighbor. If R2 arises from a belt of macroscopic bodies, then the brighter clumps might be transient phenomena arising when embedded parent bodies collide; a similar process is believed to be at work in Saturn's clumpy F ring (*18*–*21*).

Discussion. Dynamical simulations of the closely packed inner uranian moons reveal that they will perturb each other into crossing orbits at million-year time scales (*3*). The discovery of Cupid orbiting so close to Belinda substantially exacerbates the stability problem (*22*). Our new findings provide a clearer picture of this rapidly and chaotically evolving system. The largest moons show orbital changes within decades; these subtle variations can, over time, lead to orbital collisions and disruptions. So close to the Roche limit, one might expect to find one or more debris belts within this evolving system; R2 is likely to be one such example. Mab and R1 are distant enough to be unaffected by the dynamical turmoil within the Portia group, but the mysterious orbital variations of Mab suggest that it may play a part in the underlying interactions. It is as yet unclear whether related phenomena are at work within Uranus's main ring system.

The rings and smaller moons listed in Tables 1 and 2 have probably changed quite substantially since the time of the dinosaurs, and perhaps even since the time of the Roman Empire. Uranus does not host a swarm of independent moons and rings, but instead features a closely coupled dynamical system that rivals the other known ring-moon systems in its subtlety and complexity.

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REPORTS

Electrodes with High Power and High Capacity for Rechargeable Lithium Batteries

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New applications such as hybrid electric vehicles and power backup require rechargeable batteries that combine high energy density with high charge and discharge rate capability. Using ab initio computational modeling, we identified useful strategies to design higher rate battery electrodes and tested them on lithium nickel manganese oxide [$Li(Ni_{0.5}Mn_{0.5})O_2$], a safe, inexpensive material that has been thought to have poor intrinsic rate capability. By modifying its crystal structure, we obtained unexpectedly high rate-capability, considerably better than lithium cobalt oxide ($LiCoO_2$), the current battery electrode material of choice.

Rechargeable Li batteries offer the highest energy density of any battery technology, and they power most of today's portable electronics. Although most electronics require only moderately high charge/discharge rates, newer applications, such as regenerative braking in hybrid electric vehicles (HEVs), power backup, and portable power tools, require both high energy and high power density (i.e., the ability to charge and discharge very fast), which has been difficult to accomplish with Li batteries. Cathode electrodes in rechargeable Li batteries store Li^+ and electrons by the concurrent insertion of Li^+ in a crystal structure and the reduction of a transition-metal ion (*1*). Good electrode materials therefore have high reversible storage capacity for Li (to obtain long battery life per unit weight or volume of the battery) and rapid solid-state Li^+ and electron transport. Reasonable charge/discharge times require that, at room temperature, Li^+ ions can diffuse over micrometer distances in a matter of an hour (minutes for HEV technology). Hence, materials with very fast Li diffusivity are needed to produce batteries capable of satisfying high power demands in new applications. In this report, we use ab initio computational modeling to identify the basic energy barrier that limits Li^+-ion hopping in a prototypical layered electrode structure and use the insights gained from these calculations to synthesize a material with substantially better rate capability.

Many current (e.g., $LiCoO_2$ in today's batteries) and potential intercalation electrodes have the layered structure shown in Fig. 1A, consisting of layers of transition-metal cations separated from Li layers by oxygen. In this structure, Li is coordinated octahedrally by oxygens but diffuses from site to site by hopping through intermediate tetrahedral sites (Fig. 1B) (*2*). Previous work has identified the tetrahedral site as being close to the maximum-energy position along the path between octahedral sites (*3*). Because Li hops require thermal energy fluctuations, the hopping rate decreases exponentially with the energy of the activated state, and small reductions in this activation energy can lead to substantial improvement of the Li diffusion and of the rate at which an electrode can be charged or discharged. At room temperature, a reduction of activation energy of only 57 meV increases the rate of Li migration by a factor of 10 [$\exp(-57\ meV/kT) \sim 10$]. The energy required for a Li^+ ion to cross the activated

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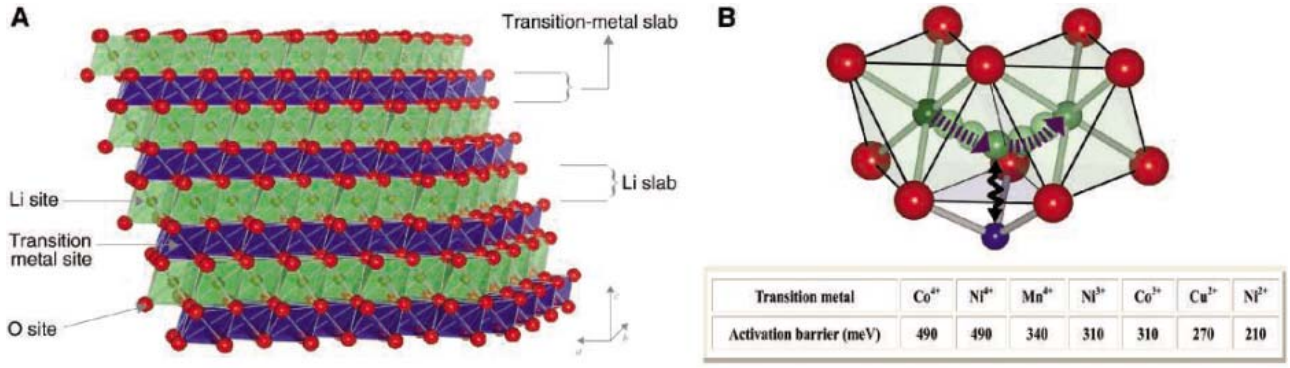

Transition metal	Co^{4+}	Ni^{4+}	Mn^{4+}	Ni^{3+}	Co^{3+}	Cu^{2+}	Ni^{2+}
Activation barrier (meV)	490	490	340	310	310	270	210

Fig. 1. (**A**) The structure of $Li(Ni_{0.5}Mn_{0.5})O_2$ consists of layers of transition metal (Ni and Mn) separated from Li layers by oxygen. In materials made by a conventional high-temperature synthesis, partial exchange of Li and Ni ions is always observed, which contracts the space through which Li can move. (**B**) Li moves from one octahedral site to another by passing through an intermediate tetrahedral site where it encounters strong repulsion from a nearby transition-metal cation. The table shows the activation barrier for Li motion for various transition metals near the activated state. Values were calculated by GGA DFT for various chemistries and Li contents.

state is likely to depend on the size of the tetrahedral site (strain effect) as well as on the electrostatic interaction between Li^+ in the activated state and the transition-metal cation directly below it (Fig. 1B). Hence, a strategy to increase Li diffusivity should focus on reducing these two contributions to the activation energy.

Using ab initio calculations performed in the generalized gradient approximation to density functional theory (GGA DFT), we can gauge the effect of both the strain and electrostatic contributions to the activation energy. The table in Fig. 1B shows the calculated energy for a Li^+ ion in the tetrahedral site with various other cations in the face-sharing octahedron adjoining it. Clearly, a low-valent transition metal such as Ni^{2+} is beneficial to the Li diffusion. The reduction from Co^{3+} (currently used) to Ni^{2+} leads to a hopping rate that is higher by a factor of 54 [$\sim\exp(100\ meV/kT)$]. Hence, a material such as $Li(Ni^{2+}_{0.5}Mn^{4+}_{0.5})O_2$, in which half the Li^+-activated states come in contact with Ni^{2+}, should have substantially higher Li diffusivity at the early stage of charging than the currently used battery material $LiCoO_2$, in which all the pathways are in contact with Co^{3+}. Because the number of high-rate pathways is well above the percolation limit, the presence of low-rate pathways (in contact with Mn^{4+}) in $LiNi_{0.5}Mn_{0.5}O_2$ should not substantially reduce the Li diffusivity. It is expected that the benefit of Ni^{2+} is noticeable until the charging level is so high that the Ni^{2+} pathways do not percolate anymore.

$LiNi_{0.5}Mn_{0.5}O_2$ is fundamentally different from the currently used electrode material, $LiCoO_2$, in which Co can only exchange one electron, and removal of all Li leads to an unstable material containing only highly oxidized Co^{4+} ions. In comparison, the transition-metal layer in $Li(Ni_{0.5}Mn_{0.5})O_2$ is bifunctional, with Ni^{2+} acting as a double redox-active center (*4*–*7*) and Mn^{4+} providing stability to the host structure (*8*). Although the distinctive electronic properties of $Li(Ni_{0.5}Mn_{0.5})O_2$ have been shown to result in high battery capacity for this material at very low charge/discharge rates (*4*, *5*, *9*, *10*), this capacity advantage over current electrode materials completely disappears at commercially interesting rates, in apparent contradiction to the predictions made based on the calculations (Fig. 1B). Our calculations were performed for materials with an ideal layered structure. However, in all $Li(Ni_{0.5}Mn_{0.5})O_2$ materials synthesized thus far, perfect separation between Li and transition-metal cations into alternating layers could not be achieved, with all materials exhibiting 8 to 12% exchange of Li and Ni (*4*, *5*, *9*, *10*). Calculations were, therefore, performed to explore the effect of Li/Ni site exchange on the Li mobility. Figure 2 shows ab initio computations of the activation energy as a function of the distance between the oxygen layers on each side of the plane of Li^+ ions (Fig. 2). As this layer-to-layer distance grows, the space in the activation site increases. It is clear that more space between these oxygen layers substantially reduces the activation energy for Li motion. These calculations also clearly point to the Li/Ni disorder as the reason $Li(Ni_{0.5}Mn_{0.5})O_2$ has not yet lived up to its high rate potential. The solid lines show the calculated O-O interlayer spacing for a material with 8.3% of Li-Ni site disorder. The dashed lines give the equivalent spacing for a hypothetical material with no Li/Ni disorder. Our calculations indicate that the Li slab space reduces from 2.64 to 2.62 Å when 8.3% of transition metal is present in the Li layer. Li motion is so sensitive to the spacing between oxygen layers that this very small change ($\sim$0.02 Å) results in a 20- to 30-meV increase in the activation barrier. More important, Li/Ni disorder greatly limits the opening of the Li slab space upon delithiation (i.e., on charging the battery). The Li slab space expands during the early stages of delithiation as a result of the removal of O^{2-}-Li^+-O^{2-} bonds across the slab, leading to faster Li motion. Whereas the Li slab space increases from 2.64 to 2.74 Å in a perfect layered system, calculations indicate that it only increases from 2.62 to 2.69 Å when 8.3% Ni ions are present in the Li layer. These observations clearly

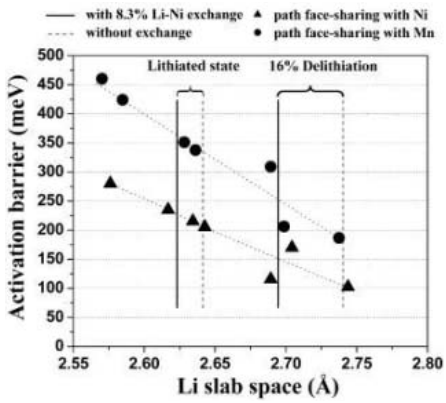

Fig. 2. Calculated activation barrier for Li migration in $Li(Ni_{0.5}Mn_{0.5})O_2$ as a function of the Li slab space. Triangles and circles represent the activated state that face-shares with Ni and Mn, respectively. The activation barriers have been calculated for a hypothetically perfect layered system, for a system with 8.3% excess Ni present in the Li layer without a change in the transition-metal layer, and for a system with 8.3% Li-Ni exchange.

indicate that the diffusivity of Li should be greatly improved by reducing the Li/transition-metal (Ni) exchange in $Li(Ni_{0.5}Mn_{0.5})O_2$, a fact that has already been noticed experimentally in $LiNiO_2$ and $LiTiS_2$ compounds (*11*, *12*).

Although Li-containing materials prepared through traditional high-temperature synthesis routes contain substantial Li/Ni disorder and are, therefore, unlikely to be high-rate electrodes, better ordering can be expected in $Na(Ni_{0.5}Mn_{0.5})O_2$ because the larger size difference between Na^+ and Ni^{2+} or Mn^{4+} creates a larger driving force for separating the alkali ions and the transition-metal ions in discrete layers. We have previously shown that the driving force for layering is likely to be increased if a larger ion, such as Na^+, is used instead of Li^+ because of the increased size mismatch between Na^+ and transition-metal ions such as Ni^{2+} (*13*). Na compounds can be trans-

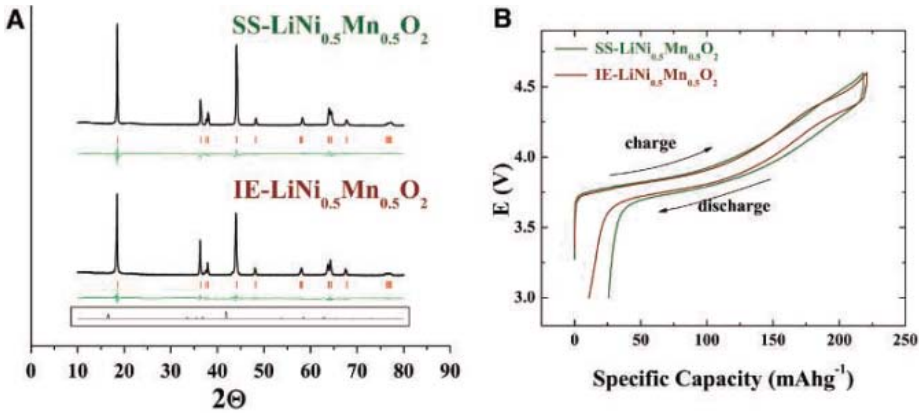

Fig. 3. (**A**) XRD patterns of SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 (top) and IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 (bottom). The observed pattern, the calculated peak positions, and the difference between the two patterns are shown for each XRD pattern. The bottom inset is the XRD pattern of the Na precursor. The precursor peak is not observed after ion exchange. The Rietveld refinement of SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 (in R-3m) gives c = 14.2820(14) Å, a = 2.8850(1) Å, and z = 0.25736(37); the Ni-Li exchange = 10.9%, with R_p = 13.6 and R_{wp} = 15.3. The refinement of IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 gives c = 14.3437(8) Å, a = 2.8924(1) Å, and z = 0.25907(21); the Ni-Li exchange = 4.3%, with R_p = 7.08 and R_{wp} = 8.74. (**B**) First charge/discharge curves of IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 and SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 within the voltage window of 3.0 to 4.6 V at C/20 rate.

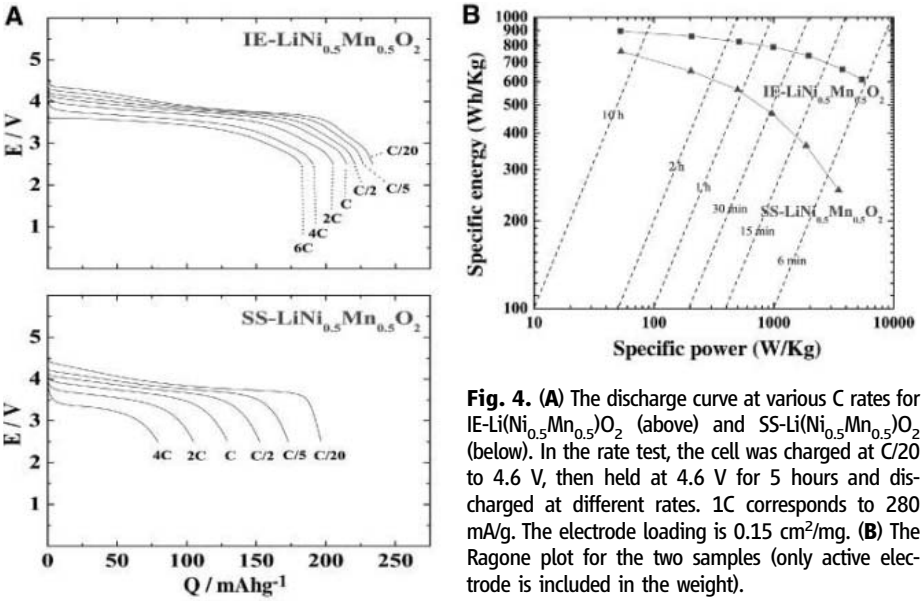

Fig. 4. (**A**) The discharge curve at various C rates for IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 (above) and SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 (below). In the rate test, the cell was charged at C/20 to 4.6 V, then held at 4.6 V for 5 hours and discharged at different rates. 1C corresponds to 280 mA/g. The electrode loading is 0.15 cm^2/mg. (**B**) The Ragone plot for the two samples (only active electrode is included in the weight).

formed to well-layered Li compounds by ion exchange of Na^+ for Li^+ (*14*–*16*). Ion exchange is a soft chemical approach performed at relatively low temperature so that only Na^+ is replaced by Li^+, keeping the rest of the structure intact. We have indeed obtained a well-layered material with excellent performance by ion-exchanging Li for Na in Na($Ni_{0.5}Mn_{0.5}$)O_2. Details of the synthesis procedure are given in the Supporting Online Material (*17*).

The x-ray diffraction (XRD) pattern of the Li($Ni_{0.5}Mn_{0.5}$)O_2 obtained by ion exchange [IE-Li($Ni_{0.5}Mn_{0.5}$)O_2] is shown in Fig. 3A. For comparison, we also show Li($Ni_{0.5}Mn_{0.5}$)O_2 synthesized through a conventional solid-state reaction [SS-Li($Ni_{0.5}Mn_{0.5}$)O_2]. The refined structural parameters for SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 are in good agreement with the literature (*4*, *9*, *10*, *18*–*20*). Noticeably, there is a substantial increase in the c-lattice parameter from 14.28 Å in SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 to 14.34 Å in IE-Li($Ni_{0.5}Mn_{0.5}$)O_2. The c-lattice parameter is perpendicular to the layers in the structure of Fig. 1, and its increase signifies an increase in the space around the Li layer. Defining the Li slab space as the average distance between the oxygen layers around the Li layer, the Li slab space increases from 2.59 Å in SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 to 2.65 Å in IE-Li($Ni_{0.5}Mn_{0.5}$)O_2. Given the computational results in Fig. 2, IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 should therefore have a substantially higher Li diffusivity. The small quantitative disagreement with the calculated slab spaces is typical for modern ab initio approaches. No Na precursor peaks are visible in the XRD, and only about 0.3 weight percent of Na could be detected by inductively coupled plasma (ICP) measurement in IE-Li($Ni_{0.5}Mn_{0.5}$)O_2, which implies that the ion exchange of Na and Li is complete.

Rietveld refinement of the XRD gives Li/Ni exchanges of 10.9% and 4.3%, respectively, in the SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 and IE-Li($Ni_{0.5}Mn_{0.5}$)O_2. Because the site occupancies obtained by Rietveld refinement can be somewhat inaccurate as a result of preferential texture of the sample, and when refining occupancies for multiple cations on the same crystallographic site, the Li/Ni exchange was independently verified by solid state ^{6}Li magic angle spinning nuclear magnetic resonance (MAS NMR). Quantitative analysis of the 1450–parts per million peak in the NMR spectrum, which is known to correspond to Li in a transition-metal site (*21*), reveals that Li-Ni exchange is even lower, about 0.5% (*17*). This suggests that our strategy to obtain a better layered structure with larger slab space is successful.

Electron microscopy revealed plate-shaped crystals of about 1 μm for IE-Li($Ni_{0.5}Mn_{0.5}$)O_2, whereas the SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 forms cubic particles of about 0.5 μm (*17*). The anisotropic shape of the crystallites offers further evidence for the more layered structure of IE-Li($Ni_{0.5}Mn_{0.5}$)O_2.

Figure 3B shows the first charge and discharge profiles of IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 and SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 tested in an electrochemical cell at a rate corresponding to fully charging the theoretical capacity of the material in 20 hours (C/20). Whereas the charge/discharge behavior of IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 is very similar to that of SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 below 4 V, the plateau at 4.3 V is more pronounced in IE-Li($Ni_{0.5}Mn_{0.5}$)O_2. Because the 4.3-V plateau is observed at about x = 0.6 to 0.7 [Li_{1-x}($Ni_{0.5}Mn_{0.5}$)O_2], it could be caused by Li-vacancy ordering (*22*). The absence of transition metals in the Li layer is likely to enhance Li-vacancy ordering.

Figure 4A shows that, despite its larger particle size, the rate capability of IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 is superior to that of SS-Li($Ni_{0.5}Mn_{0.5}$)O_2. Although the performance of the materials is similar at low rates, IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 retains much higher capacity at high rates than does SS-Li($Ni_{0.5}Mn_{0.5}$)O_2. Even at a 6C rate (one charge of 280 mAh/g in 10 min), IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 delivers a discharge capacity of 183 mAh/g. There is no published data available for a rate as high as 6C, but comparison with the best electrochemical data published for this material so far [135 mAh/g at a 397 mA/g rate (*9*)] confirms that we have developed a material with substantially improved performance.

For practical applications, the trade-off between power (rate) and energy density is important and is often represented in a Ragone plot (Fig. 4B). Most Li-battery materials show a substantial decrease in specific energy as one draws more current from them, making them less useful in applications such as EV (electric vehicle), HEV, and power tools, where high charge and

discharge rates are required. Figure 4B shows that IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 clearly retains its energy storage capacity even at the very high rate required for those applications. At a 6-min charge/discharge rate, IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 delivers almost double the energy density of SS-Li($Ni_{0.5}Mn_{0.5}$)O_2. Initial tests on the capacity retention with full charge/discharge cycling are promising, with a fade of 0.6% per cycle for IE-Li($Ni_{0.5}Mn_{0.5}$)O_2 versus 0.8% per cycle for SS-Li($Ni_{0.5}Mn_{0.5}$)O_2 (*17*).

In conclusion, we have used ab initio computational modeling to infer that the combined use of low-valent transition-metal cations and low strain in the activated state are key strategies for increasing the rate capability of layered cathode materials, and we have successfully synthesized Li($Ni_{0.5}Mn_{0.5}$)O_2 with very little intralayer disordering to optimize those factors. In agreement with our theoretical predictions, this material retains its capacity at high rates. Substitution of Co for Ni and Mn can also be used to reduce the Li/Ni exchange and improve rate performance (*23*, *24*), although the use of Co increases the cost and reduces the safety of the material (*25*). Although Li($Ni_{0.5}Mn_{0.5}$)O_2 displays an exciting combination of high rate and high capacity, several other factors, such as thermal stability, cycle life, and the extra cost from the ion-exchange process, will need to be further investigated before its application in commercial products can be considered. If the outcome of such development studies is positive, Li($Ni_{0.5}Mn_{0.5}$)O_2 would be a potential cathode material for high rate applications.

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www.sciencemag.org/cgi/content/full/311/5763/977/DC1
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Figs. S1 to S4
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Plasma Acceleration Above Martian Magnetic Anomalies

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Auroras are caused by accelerated charged particles precipitating along magnetic field lines into a planetary atmosphere, the auroral brightness being roughly proportional to the precipitating particle energy flux. The Analyzer of Space Plasma and Energetic Atoms experiment on the Mars Express spacecraft has made a detailed study of acceleration processes on the nightside of Mars. We observed accelerated electrons and ions in the deep nightside high-altitude region of Mars that map geographically to interface/cleft regions associated with martian crustal magnetization regions. By integrating electron and ion acceleration energy down to the upper atmosphere, we saw energy fluxes in the range of 1 to 50 milliwatts per square meter per second. These conditions are similar to those producing bright discrete auroras above Earth. Discrete auroras at Mars are therefore expected to be associated with plasma acceleration in diverging magnetic flux tubes above crustal magnetization regions, the auroras being distributed geographically in a complex pattern by the many multipole magnetic field lines extending into space.

Earth's polar aurora and related phenomena, such as magnetic and ionospheric disturbances, have been studied for well over half a century. The first proof that the aurora is caused by energetic electrons precipitating into Earth's topside atmosphere came from high-altitude sounding-rocket measurements (*1*). Electrons accelerated downward by magnetic field–aligned electric fields cause intense bright auroral arcs, often referred to as discrete auroras. Intense fluxes of nearly monoenergetic electrons (*2*) were the first evidence for magnetic field–aligned electric fields. Subsequent observations of accelerated electrons were made from polar orbiting satellites. The electrons' peak energy displayed a characteristic "inverted-V" signature in an energy-time spectrogram (*3*), which became the particle attribute of a discrete aurora. An additional proof of concept was observations of electrons and ions accelerated in opposite directions (*4*, *5*).

Inverted-V–like ion features near Mars, first reported by the Phobos-2 spacecraft (*6*), were associated with the temporal and spatial variability of the energy and momentum transfer between martian plasma and the solar wind (*7*, *8*). This was because auroras, specifically discrete auroras, are associated with magnetized planets, and no strong intrinsic magnetic fields were evident from Phobos-2 data, thus ruling out any analogy with the terrestrial aurora.

The Mars Global Surveyor (MGS) findings of crustal magnetic anomalies at Mars (*9*) considerably changed the picture. We now expect to find diverging magnetic field "cusps" above Mars (*10*) and closed magnetic loops (*11*), with local magnetic conditions similar to those found above Earth's polar region, albeit weaker and topologically different. A set of magnetic multipoles at specific longitudes and latitudes of Mars may characterize the crustal magnetization. Indeed, the first observation of auroral emission at Mars (*12*) was made above a strong crustal magnetization at 177°E and 52°S. The emissions in the 150- to 300-nm bands (CO and O) were most likely excited by high fluxes of charged particles.

Our study identified regions with downward-accelerated electrons and upward-accelerated ionospheric ions near local midnight. We studied how the acceleration regions map to magnetic cusps and clefts bound by strong magnetizations at Mars. We compared the energy spectra of accelerated electrons in the nightside of Mars with those associated with terrestrial discrete auroras. Finally, we computed the energy flux of precipitating electrons and estimated, from a

terrestrial analogy, the expected intensity of the nightside aurora at Mars.

The Analyzer of Space Plasma and Energetic Atoms (ASPERA-3) experiment (*13*) has two plasma instruments: an Eectron Spectrometer (ELS) and an Ion Mass Analyzer (IMA). The ELS provides electron measurements in the energy range from 0.001 to 20 keV, with 8% energy resolution. The intrinsic field of view is 4° × 360°. The 360° aperture is divided into 16 sectors.

The IMA provides ion measurements in the energy range from 0.01 to 30 keV for the main ion components H^+, He^{++}, He^+, and O^+ and for the group of molecular ions [$20 < M/q < \sim 80$ (M, mass; q, electric charge)]. The IMA has a 4.6° × 360° field of view. Electrostatic sweeping provides elevation (±45°) coverage for a total field of view of about 2π.

ASPERA-3 data from 33 Mars Express (MEX) traversals of the martian cavity were analyzed, most of the data being taken during a period of favorable eclipse orbits in February and March 2005. During this period, the spacecraft traversed the midnight-sector cavity. An important selection criterion was the acceleration of ions and electrons; the ionospheric ions accelerated upward and the electrons accelerated downward. The spacecraft "footprint" was mapped in geographic latitude and longitude down to a 400-km MGS "open-closed" magnetic field map.

During an inverted-V event on 20 February 2005 (Fig. 1), narrow beams of heavy ions [O^+ (30%), O_2^+ (49%), and CO_2^+ (21%)], consistent with the ionospheric composition at 220 to 250 km (*14*), were observed to flow upward/tailward while substantial fluxes of electrons moved in the opposite direction (downward/sunward). Displaced Maxwellian distributions were used for temperature calculations (that is, the temperatures were determined from the high-energy tail of the electron and ion distributions). The beams are relatively cool (Fig. 1), the beam energy being substantially higher than the thermal energy. The general characteristics of these inverted-V events are (i) the coincident existence of narrow upgoing ionospheric ion beams and energized downgoing electrons and (ii) a tendency for the downward electron acceleration to increase with decreasing altitude. These are characteristics analogous to those of auroral acceleration near Earth. The trajectory in fact maps to semi-open magnetic field lines: in the boundary between a magnetic anomaly and open field lines. It does not map

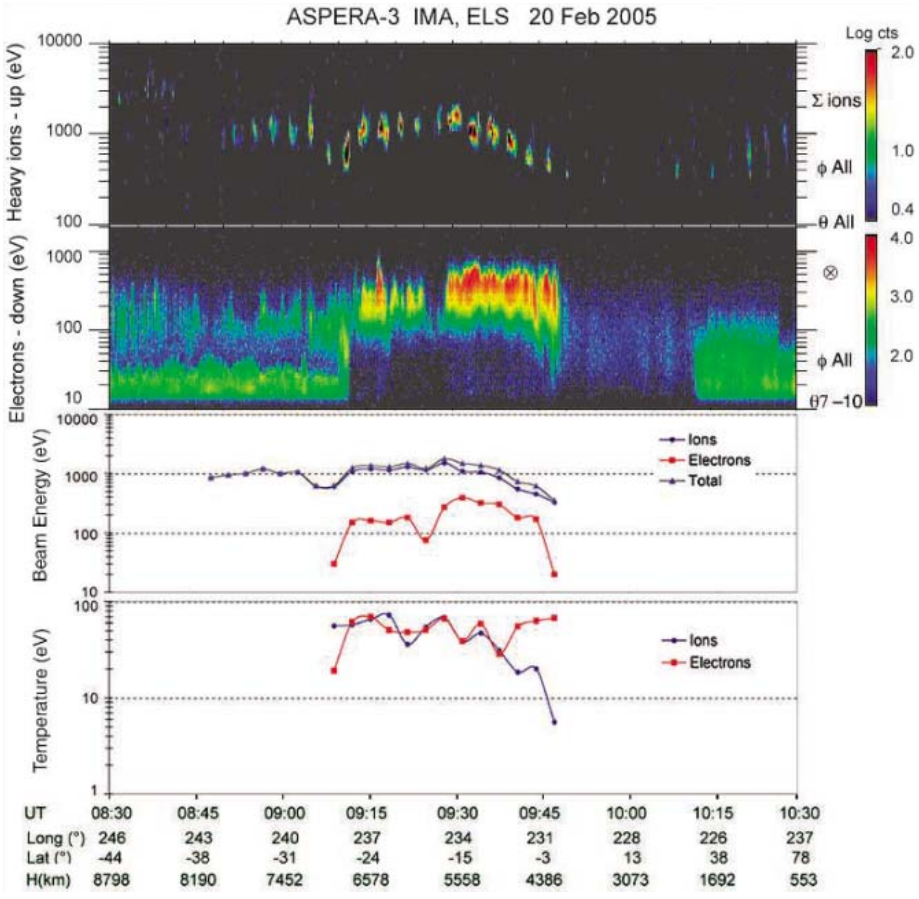

Fig. 1. Energy-time spectrogram for ions and electrons during an inverted-V event in the tail eclipse. The third panel from the top shows the acceleration energy (peak energy) and total acceleration (electrons + ions). The bottom panel shows electron and ion temperatures. Coordinates are Mars east longitude and latitude. UT, universal time; H, height.

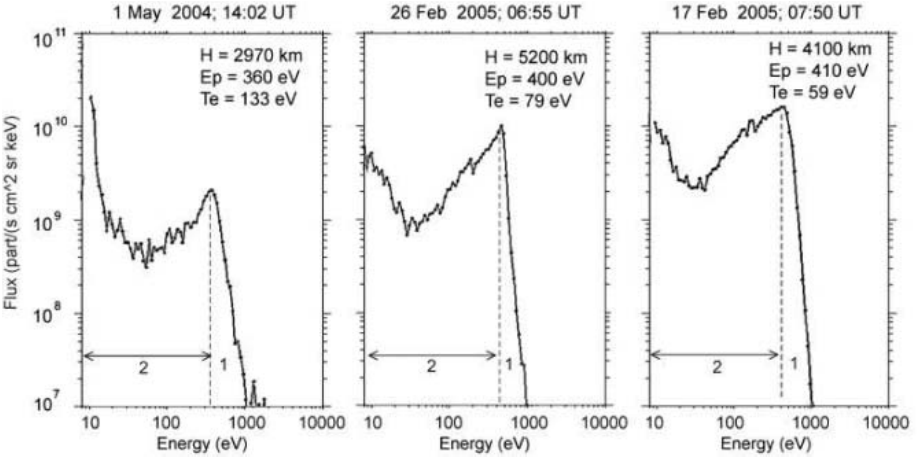

Fig. 2. Three ASPERA-ELS electron energy spectra taken at different altitudes and times, suggesting acceleration through a potential drop V_0 ($E_p = eV_0$). Te, electron temperature. The energy regimes correspond to accelerated primaries (1) and backscattered + secondary electrons (2).

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directly to the closed flux-tube magnetic anomaly. This is another analogy with Earth's auroral zone: The inverted V's are usually found in the boundary region between open and closed magnetic field lines.

Three ELS electron spectra from three separate orbits (Fig. 2) provide further evidence for auroral acceleration in a quasi-static electric potential drop. Based on an acceleration model (*15*), the characteristics of downward/parallel acceleration of electrons in a potential drop V_0 can be identified: a sharp energy peak, E_p, related to accelerated primary electrons ($E_p = eV_0$), and secondary electrons originating from reflection and backscattering above Mars. Upgoing ions form narrow monoenergetic beams; the downgoing electrons are more isotropic and less monoenergetic. These characteristics are consistent with theory (*16*) and observations (*5*) near Earth.

A good correlation is seen between ion beam energy and ion beam temperature (Fig. 3, left). The linear relation $y = 8.5x + 623$ (eV) has a correlation coefficient $R^2 = 0.76$. Studies of upward-flowing H^+ ions near Earth by the Viking satellite [figure 2.28 of (*5*)] give a similar linear relation $y = 4.9x + 210$ (eV), with $R^2 = 0.74$. The relations imply that ion heating goes together with parallel acceleration. The ion beams are cool below ≈1 keV, with the temperature increasing proportional to the parallel energy above ≈1 keV, but the beam energy remains at least a factor of 10 higher than the beam temperature. In analogy with Earth, the increased ion beam temperature may be due to a transverse (to the magnetic field) acceleration process (*17*). The electron beam energy and electron thermal energy (Fig. 3, right) also appear to be correlated, although with less significance ($R^2 = 0.58$) than the ion correlation. Electron temperatures for low-energy peaks are in the range typical for the magnetosheath (20 to 50 eV). Wave activity inferred from high-time resolution ELS data in connection with ion and electron heating (*18*, *19*) suggests that waves are the cause of the gradual electron heating.

A statistical analysis of the maximum ion inverted-V peak energy shows no altitude dependence ($R^2 = 0.15$). This implies that the acceleration process is quite variable, governed by external solar wind dynamics (pressure and interplanetary electric and magnetic fields). As for the mapping of the acceleration regions to magnetic anomalies, we find a tendency for inverted V's to occur near local midnight, the observations clustering around the mean 162°E and –7°S. The clustering of observations close to local midnight is in part due to the selection criteria (the cavity).

When the inverted-V footprints are plotted versus geographic latitude and longitude, an interesting picture emerges (Fig. 4): a clustering of data points in boundaries between open and closed field lines. Few data points fall within larger areas of open (red) or closed (black) magnetic field. We therefore conclude that the

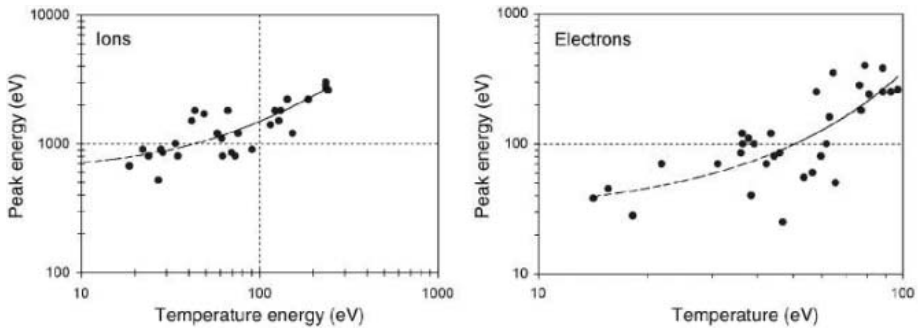

Fig. 3. Ion and electron inverted-V peak energy versus temperature in the nightside of Mars. (**Left**) Ions. The dashed line marks a linear fit with function $y = 8.5x + 623$ (eV), $R^2 = 0.76$. (**Right**) Electrons. The dashed line marks an exponential fit with function $y = 27.2 \cdot \exp(0.026x)$ (eV). $R^2 = 0.58$.

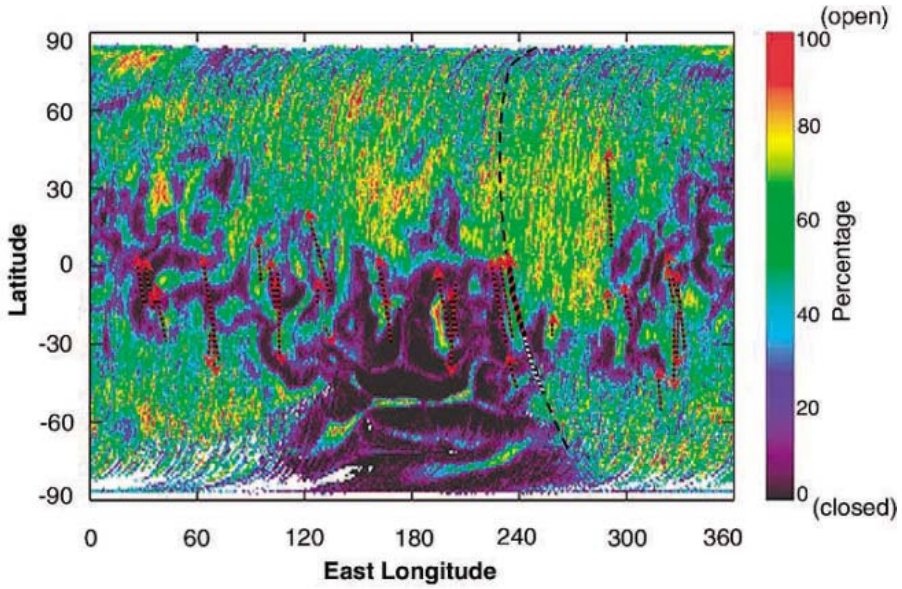

Fig. 4. Satellite ground track projections of nightside ion inverted V's and their relation to crustal magnetization. The scale at right indicates the percentage of open magnetic field lines at MGS altitudes (≈400 km), with red indicating 100% open and black 100% closed magnetic flux tubes. The satellite ground tracks are indicated by dashed lines; the red arrowheads mark the exit of the inverted V's. The black dashed line near longitude 240° marks the entire ground track of the inverted-V traversal shown in Fig. 1.

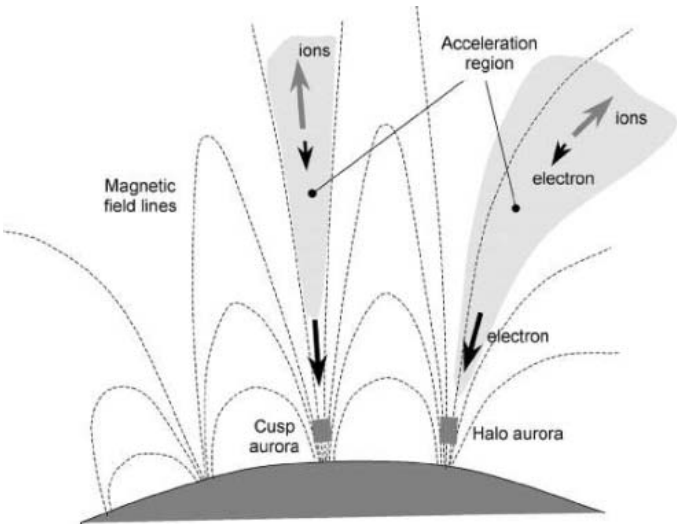

Fig. 5. Diagram of the auroral plasma acceleration above the magnetic anomalies at Mars. A cusp/cleft aurora is expected to occur between adjacent anomalies and a halo aurora to occur circumscribing the large-scale region of crustal magnetization.

inverted V's are associated with boundary regions between open and closed field lines. The precipitation of electrons and the corresponding acceleration and escape of ionospheric ions take place in clefts interfacing magnetic anomalies and in the boundary interfacing the large-scale magnetization region with the nonmagnetized region at Mars.

Results from ASPERA-3 on MEX (*20*) suggest that energization and outflow of plasma may initiate at fairly low altitudes. Similarly, the nightside energization and outflow may also reach down to low altitudes, perhaps even lower in view of the low nightside ionization. The observations of upward-accelerated ions combined with downward-accelerated electrons are observed on flux tubes that are semi-open or open, connecting to strong crustal magnetizations. However, we also observe acceleration in boundaries enclosing the large-scale regions of crustal magnetization. The intense fluxes of upgoing ionospheric ions represent the erosion of ionospheric plasma and the formation of plasma density cavities (*21*). A combination of parallel electric fields and waves deepens the cavities and promotes an acceleration process in which parallel acceleration and heating are strongly coupled. This is consistent with the linear relation found between the ion peak/beam energy and the beam temperature (Fig. 3, left), as previously observed above terrestrial discrete auroras associated with field-aligned plasma acceleration (*5*).

Discrete auroras are therefore expected to occur in clefts interfacing with strong crustal magnetization regions at Mars (Fig. 5), but also in the interface region connecting the void and presence of crustal magnetizations at Mars. To complete the analogy between terrestrial discrete auroras and martian auroras, the precipitating energy flux of electrons Θ_a is derived by adding the local energy flux Θ_i and the energy flux gained by electron acceleration P_a down to the atmosphere. P_a is computed using the acceleration voltage (V_0) inferred from upgoing ionospheric ion beams (Fig. 3, left). For electrostatic acceleration along a unit magnetic flux tube, assuming acceleration in a narrow altitude range, the total energy flux gain is given by $P_a \approx eV_0\Phi_i$, where Φ_i represents the local downward electron flux. Thus $\Theta_a \approx \Theta_i + eV_0\Phi_i$. From 17 inverted V's, we obtain the local maximum energy flux Θ_i = 0.01 to 3 mW/m^2, from which we derive Θ_a = 1 to 50 mW/m^2. The latter corresponds to 2- to 80-kilorayleigh visible emissions (at 557.7 nm; the "green line") above Earth. In a similar manner, the density distribution of atomic oxygen from a time-averaged model of the martian atmosphere (*22*) suggests that the green-line aurora is generated above magnetic cusps and clefts in the nightside of Mars at atmospheric heights of 50 to 80 km.

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23. ASPERA-3 on the European Space Agency's (ESA's) MEX is a joint effort between 15 laboratories in 10 countries. We are indebted to the national agencies supporting ASPERA-3 and to ESA for making MEX a great success.

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Dissociation of $MgSiO_3$ in the Cores of Gas Giants and Terrestrial Exoplanets

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$CaIrO_3$-type $MgSiO_3$ is the planet-forming silicate stable at pressures and temperatures beyond those of Earth's core-mantle boundary. First-principles quasiharmonic free-energy computations show that this mineral should dissociate into CsCl-type MgO + cotunnite-type SiO_2 at pressures and temperatures expected to occur in the cores of the gas giants and in terrestrial exoplanets. At ~10 megabars and ~10,000 kelvin, cotunnite-type SiO_2 should have thermally activated electron carriers and thus electrical conductivity close to metallic values. Electrons will give a large contribution to thermal conductivity, and electronic damping will suppress radiative heat transport.

The transformation of $MgSiO_3$-perovskite into the $CaIrO_3$-type structure near Earth's core-mantle boundary (CMB) conditions (*1–3*) invites a new question: What is the next polymorph of $MgSiO_3$? The importance of this question has increased since the discoveries of two new exoplanets: the Earth-like planet with ~7 Earth masses (*4*) (Super-Earth hereafter) and the Saturn-like planet with a massive dense core with ~67 Earth masses (*5*) (Dense-Saturn hereafter). The extreme conditions at the giants' cores (*6*) and exoplanet interiors are challenging for first-principles methods. Electrons are thermally excited, and core electrons start to participate in chemical bonds. This requires either all-electron methods or the development of pseudopotentials based on core orbitals. Neither density functional theory (DFT) nor the quasiharmonic approximation (QHA) have been tested at these ultrahigh pressures and temperatures (*PT*s). Here, we use the Mermin functional (*7*), i.e., the finite electronic temperature (T_{el}) version of DFT that includes thermal electronic excitations, and ultrasoft pseudopotentials (*8*) based on orbitals with quantum number n = 2 and 3 for all three atoms. We studied $MgSiO_3$, MgO, and SiO_2 up to 80 Mbar and 20,000 K (figs. S1 and S2, A to C).

$MgSiO_3$ could transform to another ABX_3-type silicate or dissociate. We searched systematically for possible ABX_3 structures having likely high-pressure coordinations and connectivities, but found none with enthalpy lower than the $CaIrO_3$-type polymorph (see supporting online material). This phase dissociated into CsCl-type MgO and cotunnite-type SiO_2 at 11.2 Mbar in static calculations (Fig. 1). Both binary oxides undergo phase transitions below 11.2 Mbar. MgO undergoes the NaCl-type → CsCl-type transformation at 5.3 Mbar, and SiO_2 undergoes a series of phase transitions: stishovite → $CaCl_2$-type → α-PbO_2-type → pyrite-type → cotunnite-type at 0.48, 0.82, 1.9, and 6.9 Mbar, respectively (Fig. 2). Our static transition pressures agree well with previous first-principles results (*9–12*) and experimental transition pressures (*13*, *14*), except for the α-PbO_2-type → pyrite-type transition in SiO_2, which has been observed once at 2.6 Mbar (*15*). CsCl-type MgO and cotunnite-type SiO_2 have not yet been seen experimentally. Baddeleyite-type and OI-type phases occur as pre-cotunnite phases in TiO_2 (*16*), an analog of SiO_2. Our results show

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that, in agreement with previous calculations (*12*), these phases are metastable with respect to pyrite-type and cotunnite-type SiO_2. Phonon frequencies in the $CaIrO_3$-type phase and in the binary oxides increase with pressure. In our calculations, no soft phonons occurred up to 80 Mbar, the pressure at Jupiter's center. As expected, soft phonons occurred in CsCl-type MgO (~2 Mbar) and in cotunnite-type SiO_2 (~1.5 Mbar) upon decompression (fig. S2).

Thermal electronic excitations have negligible effect on the structural, vibrational, and thermal properties of these phases, even at 20,000 K, shifting the phase boundary by less than 1 GPa. Empirically, the QHA should work well until the thermal expansivity $\alpha(P,T)$ becomes nonlinear (*17*). We find linear T scaling up to the dashed lines in the phase diagram shown in Fig. 3A. The Clapeyron slope ($dP/dT = dS/dV$) of the dissociation has large negative values at most pressures: −18 MPa/K at 5000 K increasing to −33 MPa/K at 10,000 K. This is caused by an increase in the average bond lengths [2.91 to 3.08 atomic units (au) for Mg-O bond and 2.59 to 2.76 au for Si-O bond] across the dissociation as cation coordination numbers increase. This decreases the average phonon frequencies and increases the entropy (*18*). At the same time, there is a density increase of 1 to 3% (Fig. 3B; fig. S3 and table S2). Negative Clapeyron slopes occurred also for the NaCl-type to CsCl-type MgO and for the pyrite-type to cotunnite-type SiO_2. In both cases, cation coordination numbers and average bond lengths increase through the transition (3.10 to 3.33 au in NaCl-type → CsCl-type MgO; 2.81 to 2.90 au in pyrite-type → cotunnite-type SiO_2).

This dissociation should affect models of the gas giants' cores (Fig. 3). $CaIrO_3$-type $MgSiO_3$ cannot exist in the cores of Jupiter and Saturn, but should survive in the cores of Uranus and Neptune, unless another phase transition not identified yet occurs at lower pressures. In Jupiter and Saturn, the dissociation occurs at *PT*s typically expected within the metallic-hydrogen envelope. The importance of this transformation for terrestrial exoplanets is more striking. Super-Earth is predicted to have a temperature of at least ~4000 K and a pressure of ~10 Mbar at its CMB (*19*). This places the dissociation near its CMB. The eventual occurrence of this endothermic transition with a large negative Clapeyron slope just above its CMB would be similar to the occurrence of the endothermic postspinel transition near the core of Mars. Geodynamical modeling suggests that this might be the cause of a proposed large martian superplume (*20*). Convection in a Dense-Saturn planet could be dramatically affected. *PT*s in this planet should be higher than in Saturn's interior (Fig. 3), given its smaller size and higher surface *T*s. A transformation with such large negative Clapeyron slope in the middle of the silicate core/mantle of terrestrial planets is likely to inhibit convection (*21*), promote layering, and produce differentiated mantles/cores, with a lower layer consisting primarily of oxides.

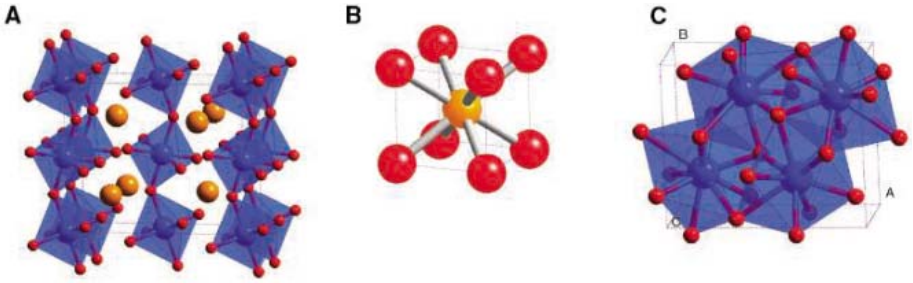
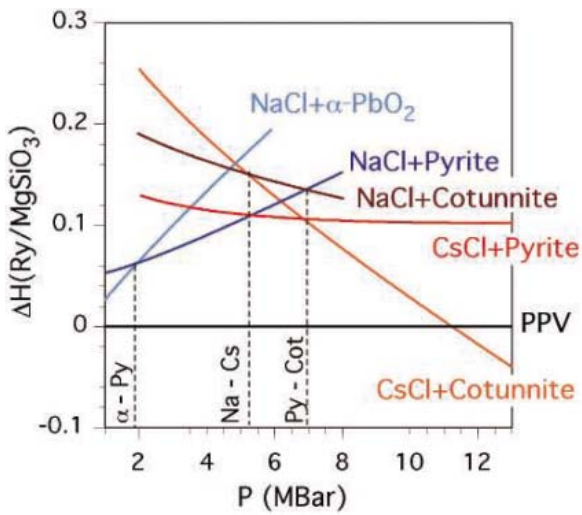


Fig. 1. Crystal structures of the stable phases. (**A**) $CaIrO_3$-type $MgSiO_3$ at static 10 Mbar. The space group is *Cmcm*. Lattice constants are (*a*, *b*, *c*) = (2.10, 6.42, 5.26) Å. Atomic coordinates are Mg(4c) (0, 0.749, 0.25), Si(4a) (0, 0, 0), O_1(4c) (0, 0.070, 0.25), and O_2(8f) (0, 0.353, 0.438). (**B**) CsCl-type MgO at static 12 Mbar. The space group is *Pm3m*. The lattice constant is a = 1.870 Å. (**C**) Cotunnite-type SiO_2 at static 12 Mbar. The space group is *Pbnm*. Lattice constants are (*a*, *b*, *c*) = (4.69, 3.95, 2.08) Å. Atomic coordinates are Si(4c) (0.141, 0.232, 0.25), O_1(4c) (0.435, 0.348, 0.25), and O_2(4c) (0.666, 0.984, 0.25).

Fig. 2. Differences between static enthalpies of aggregation of MgO and SiO_2 in various forms and $CaIrO_3$-type $MgSiO_3$. Dashed lines denote static transition pressures of NaCl-type → CsCl-type MgO, α-PbO_2-type → pyrite-type SiO_2, and pyrite-type → cotunnite-type SiO_2.



At *PT*s relevant for the giants and exoplanets, major changes in materials properties take place: These minerals become intrinsic semiconductors with electronic gaps (Fig. 4A). Local density approximation (LDA) usually underestimates band gaps by ~50%, whereas electron-phonon interactions cause gaps to narrow by a couple of eVs at elevated *T*s (*22*). The intrinsic carrier [electrons (n) and holes (p)] concentrations, in the range of 10,000 to 20,000 K (Fig. 4B), are typical of semimetals or heavily doped semiconductors. We focus on cotunnite-type SiO_2 with the largest carrier concentration.

In evaluating transport coefficients, we treat holes as immobile (*23*). This model is motivated by the relatively flat valence band edge of cotunnite-type SiO_2. Only thermal electrons are free and can carry both electrical and heat currents. The carrier density, n, from Fig. 4B can be represented by

$$n = 4\left(\frac{1}{V_c \lambda_{th}^3}\right)^{1/2} e^{-E_g/2k_BT} \qquad (1)$$

where the thermal wavelength is $\lambda_{th} = \sqrt{2\pi\hbar^2/m_e k_B T}$, and the cell volume V_c and effective mass m_e are 276 a_0^3 and 0.4m, with a_0 and m being the Bohr radius and the electron mass. Assuming that band gap E_g = 5 eV and taking $T = 10^4$ K, we obtain $n \approx 8 \times 10^{20}$ cm^{-3}; 0.9% of SiO_2 units have one excited electron and hole. This carrier density is typical of semimetals or heavily doped semiconductors.

The electrical conductivity, σ, is obtained from (*24*, *25*)

$$\sigma = ne\mu \text{ and } \mu = \frac{e\langle\tau\rangle}{m} \qquad (2)$$

where μ is the mobility and $\langle\tau\rangle$ is the average inverse scattering rate. There are three scattering mechanisms: (i) Coulomb scattering of carriers from each other; (ii) scattering from impurities or defects; and (iii) scattering by phonons, both Fröhlich (F) and optical deformation potential (*24*). Coulomb scattering is primarily electrons scattering from holes. Be-

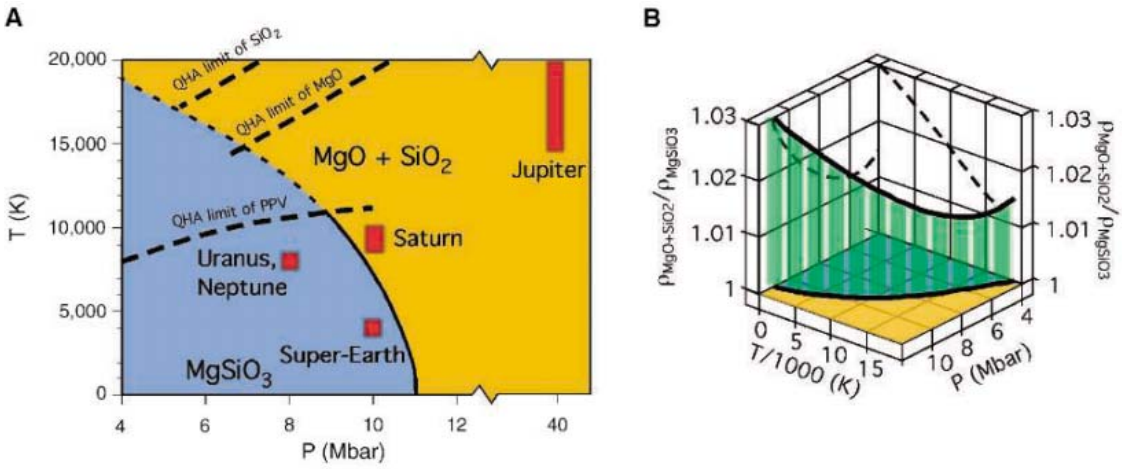


Fig. 3. (**A**) Pressure-temperature phase diagram showing the dissociation of $CaIrO_3$-type $MgSiO_3$ into CsCl-type MgO and cotunnite-type SiO_2. Red areas denote estimated pressure-temperature conditions at core-envelope boundaries in the solar giants and in Super-Earth. Dashed lines indicate the limit of validity of the quasiharmonic approximation (QHA). The dashed part of the phase boundary is more uncertain. (**B**) Density increase caused by the dissociation.

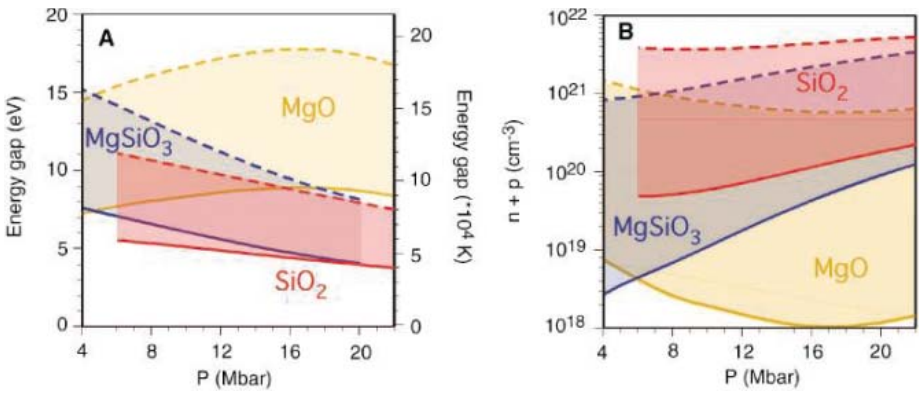


Fig. 4. (**A**) Solid and dashed lines denote the LDA band gaps of the phases involved, E_g^{LDA} and $2 \times E_g^{LDA}$, respectively. Actual band gaps should be in this range. (**B**) Total carrier (n = electrons, p = holes) concentration at 10,000 K (solid lines) ~ 20,000 K (dashed lines) assuming $E_g = 2 \times E_g^{LDA}$. This should be a lower bound for the carrier concentration.

cause holes are assumed to be localized, this is just a form of charged impurity scattering and likely weaker than scattering from impurities (Al, Fe, OH). Impurity and Fröhlich scattering suffer Debye-Hückel screening (*24*, *25*), with inverse screening length κ given by $\kappa^2 = \frac{4\pi n e^2}{\varepsilon_\infty k_B T}$. With our computed dielectric constant $\varepsilon_\infty \approx 4$, $1/\kappa \sim 4.7$ Å. We obtain the following estimates (see supporting online material):

$$\frac{\hbar}{\langle \tau_{DP} \rangle} = 0.07 \times \left(\frac{T}{10^4\ \mathrm{K}} \right)^{3/2} \mathrm{eV} \quad (3)$$

$$\frac{\hbar}{\langle \tau_{F} \rangle} = 0.08 \times \left(\frac{T}{10^4\ \mathrm{K}} \right)^{1/2} \mathrm{eV} \quad (4)$$

$$\frac{\hbar}{\langle \tau_{imp} \rangle} = 7.7 x_{imp} \times \left(\frac{T}{10^4\ \mathrm{K}} \right)^{-3/2} \mathrm{eV} \quad (5)$$

where x_{imp} is the fraction of Si atoms substituted by impurities of effective charge $Z = 1$. The Fröhlich and impurity rates have additional weak T-dependence (not shown) arising from temperature-dependent screening.

The ratio between impurity and electron-phonon scattering rates is $\frac{7.7 x_{imp}}{0.15} = 50 x_{imp}$, at $T = 10^4$ K. If more than 2% of Si atoms are replaced by charged impurities, impurity scattering dominates but falls rapidly at higher T. Minor element partitioning between MgO and SiO_2 at these conditions is unknown, and impurity centers may provide thermally inexpensive sources of new carriers. Therefore, any estimate of the influence of impurities has significant uncertainty. Ignoring impurity scattering, the electron-phonon scattering then gives an upper bound for the electronic mobility $\mu \sim 20$ cm²/V·s. This exceeds mobilities of typical metals (near 10 cm²/V·s at 300 K and falling as $1/T$) but is smaller than $\mu \sim 1400$ cm²/V·s for electrons in intrinsic Si at 300 K. The result is $\sigma \approx 2.6 \times 10^3$ (ohm·cm)$^{-1}$. The corresponding resistivity, $\rho \approx$ 380 microhm·cm, is only twice that of liquid iron at atmospheric pressure (*26*). We believe this is a reliable lower limit and that uncertainties (primarily the value of E_g, the concentration of charged impurity centers, and neglect of some weaker phonon-scattering processes) may increase the resistivity by less than a factor of 5 (see supporting online material). This would still leave the material essentially a metal!

The thermal conductivity can be estimated using an appropriate Weidemann-Franz ratio, $\kappa_{el}(T) = \left(\frac{2k_B^2 T}{e^2} \right) \sigma \approx 40$ W/mK. This is large compared to values of 2 to 4 W/mK that are representative of vibrational heat transport in hot anharmonic insulators (*27*). We also conclude that radiative heat transport should not be significant, despite the high energy content in the black-body spectrum at such high T. Because of free electrons (*28*), no photons propagate with frequencies less than the plasma frequency $\omega_p = \sqrt{4\pi n e^2 / \varepsilon_\infty m_e} \approx 0.9$ eV. Above this energy, electronic absorption from deep levels in the gap, resulting from structural defects or impurities, such as iron, is likely to limit the radiative term to values smaller than the electronic contribution. The conductivities, both thermal and electrical, of cotunnite-type SiO_2 and most oxides and silicates in terrestrial exoplanets will be large because of carriers activated over the insulating gap.

The dissociation of $CaIrO_3$-type $MgSiO_3$ stands as a challenge to be investigated by ultrahigh-pressure experiments. An alternative, low-pressure analog is $NaMgF_3$. This compound exists in the *Pbnm*-perovskite structure at ambient pressure and is predicted to transform to the postperovskite structure at 17.5 GPa and to dissociate into NaF (CsCl-type) and MgF_2 (cotunnite-type) at ~40 GPa, after the fluorides undergo transitions similar to those of the oxides (*29*). The postperovskite transformation appears to have been observed already (*30*), although the dissociation has not been observed yet.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5763/983/DC1
Methods
SOM Text
Figs. S1 to S4
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References and Notes

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Changes in the Velocity Structure of the Greenland Ice Sheet

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Using satellite radar interferometry observations of Greenland, we detected widespread glacier acceleration below 66° north between 1996 and 2000, which rapidly expanded to 70° north in 2005. Accelerated ice discharge in the west and particularly in the east doubled the ice sheet mass deficit in the last decade from 90 to 220 cubic kilometers per year. As more glaciers accelerate farther north, the contribution of Greenland to sea-level rise will continue to increase.

The contribution of the Greenland Ice Sheet to sea level is a problem of considerable societal and scientific importance. Repeat-pass airborne laser altimetry measurements (*1*) showed that the ice sheet is nearly in balance in the interior but its periphery is thinning, with deterioration concentrated along the channels occupied by outlet glaciers (*2*). The most recent surveys revealed that the mass loss from the periphery is increasing with time, with approximately half of the increase caused by enhanced runoff and half by enhanced glacier flow (*3*).

Although these airborne surveys crisscrossed a large fraction of Greenland, they left major gaps in glacier coverage, particularly in the southeast and northwest. The mass loss from nonsurveyed glaciers was estimated using an ice melt model, thereby assuming no temporal changes in ice flow. If glacier dynamics is an important factor, the contribution to sea level from Greenland is underestimated using this approach. To address this issue and understand the exact partitioning between surface mass balance and ice dynamics, it is essential to estimate glacier discharge and its variability over time.

Here, we measure glacier velocities using satellite radar interferometry data collected by Radarsat-1 in fall 2000 (*4*, *5*) along the entire coast of Greenland except the southwest (Fig. 1) and repeatedly in spring and summer 2005 along selected tracks covering major glaciers. We also use European Remote Sensing satellites ERS-1 and ERS-2 data from winter 1996 in the north, east, northwest, and central west, and Envisat Advanced Synthetic Aperture Radar (ASAR) data from summer 2004 in the southwest. Ice velocity is measured with a precision of 10 to 30 m/year depending on satellite, data quality, and processing and is combined with ice thickness to calculate ice discharge.

Ice thickness is estimated with a precision of 10 m from airborne radio echo sounding data collected in 1997 to 2005 (*6*). Although grounding-line thicknesses of glaciers extending into floating ice tongues in the north are well known, ice thickness is difficult to measure at the fronts of calving glaciers in other parts of Greenland where no floating ice tongues develop. Ice thickness is only known several km upstream of the ice fronts. Ice fluxes are thus calculated at these upstream flux gates with a precision of 4%. Ice-front discharge is deduced from the upstream flux by subtracting a zero-anomaly surface mass balance (*7*) between the flux gate and the ice front. The correction is small (Table 1). Ice-front discharge is initially calculated for 1996 if data are available; otherwise, it is calculated for 2000. Ice-front discharge in subsequent years is obtained by multiplying the reference discharge by the percentage velocity increase averaged at the ice front, with a precision reduced to 10% because ice thickness is assumed to be steady. This approach alleviates the lack of frontal thickness data, accounts for higher dynamic losses nearer to the ice fronts, but omits dynamic losses below flux gates in the reference-year calculation. Mass loss for each glacier system is deduced from the ice-front discharge in excess of the zero-anomaly surface mass balance calculated for the entire drainage, with a precision of 14% (Table 1).

We examined the seasonal variability in flow speed of major glaciers in fall 2000. We found no velocity change from September to January at the 1% level over the 24-day averaging period of Radarsat-1. On the Petermann Glacier (1 in Fig. 1), a continuous set of observations in 2004 reveals an 8% increase in the summer months compared to winter (Fig. 2A). A similar seasonality is detected on Nioghalvfjerdsbrae and all southeast Greenland glaciers and has been observed on Jakobshavn Isbrae (*8*) and Columbia Glacier, Alaska (*9*). Winter velocities are therefore only 2% lower than the annual means, and flow changes must exceed 8% to be significant. No seasonal correction is applied to our data to compensate for the fact that surface velocities may represent 97 to 99% of vertically integrated velocities at the flux gates.

A nearly comprehensive estimate of ice discharge around Greenland is obtained for year 2000, and partial coverage for 1996 and 2005. The results are used to detect changes in ice discharge around the periphery caused by ice dynamics alone and determine their impact on ice sheet mass balance, independent of temporal changes in surface mass balance, i.e., accumulation and melt.

Many changes in velocity are observed in the north, but they are of little consequence to total mass balance. Harald Moltke Glacier was surging in 2005 after a quiescent phase. Nearby Tracy and Heilprin glaciers accelerated 40% and 18% in 2000 to 2005 (Fig. 2L), but the corresponding mass loss is small. Petermann Glacier has been stable since 1996, and its mass balance remains slightly negative. Academy Glacier tripled its speed in 2005 (Fig. 2C), which is typical for northern Greenland surge-type glaciers; its mass balance averages zero over the last decade. Farther east, the mass losses from decelerating Nioghalvfjerdsbrae and accelerat-

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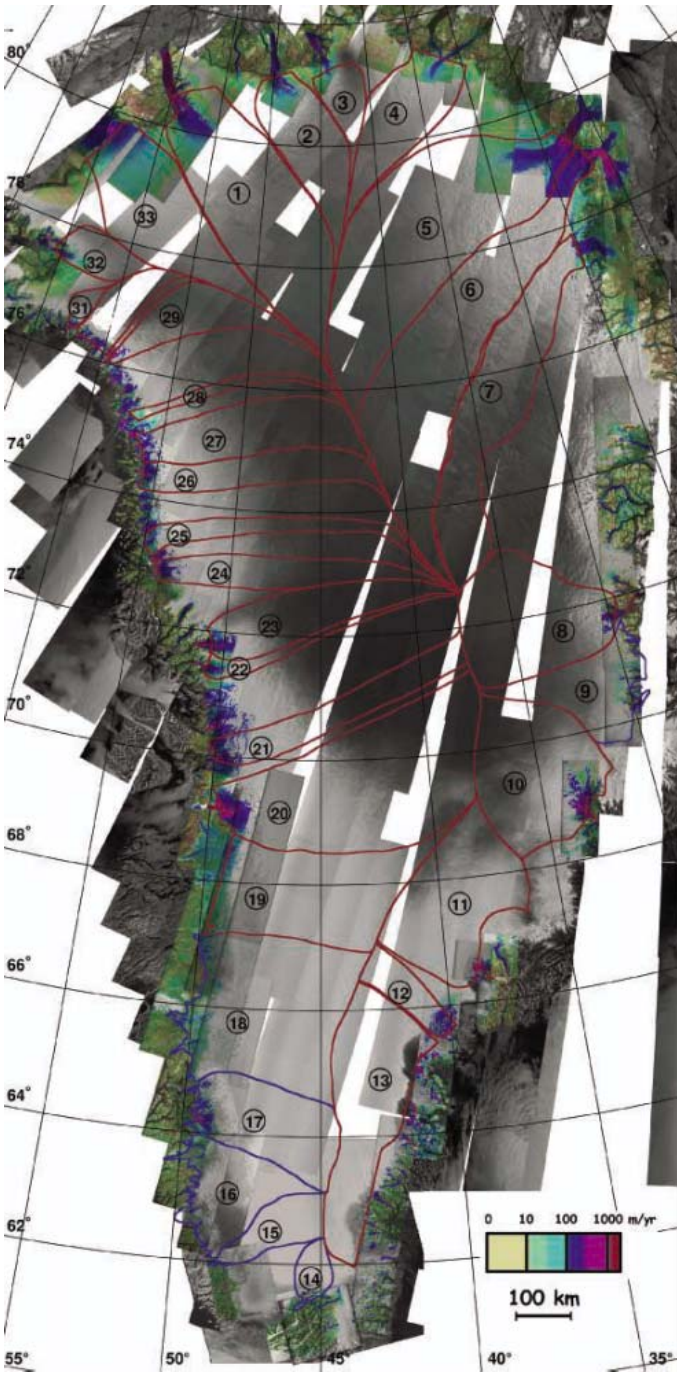

Fig. 1. Ice-velocity mosaic of the Greenland Ice Sheet assembled from year 2000 Radarsat-1 radar data, color coded on a logarithmic scale from 1 m/year (brown) to 3 km/year (purple), overlaid on a map of radar brightness from ERS-1/Radarsat-1/Envisat. Drainage boundaries for flux gates in Table 1 are in red. Drainage boundaries with no flux estimates but discussed in the text are in blue. Numbers refer to drainage basins in Table 1.

ing Zachariae Isstrøm compensate for the mass gain of decelerating Storstrømmen, a surge-type glacier in a quiescent mode (Fig. 2D). Overall, the northern sector exhibits a small mass loss (Table 1).

In central east Greenland, no flow change is detected on Daugaard-Jensen (Fig. 2E) and Vestfjord glaciers (area 9) in 1996 to 2005. The 3.7-km/year frontal speed of Daugaard-Jensen is identical to that measured in 1969 (*10*), and the glacier is in balance. Immediately south, Kangerdlugssuaq Glacier has been stable in speed since 1962, but was thinning and losing mass in 1996 (*11*). The glacier accelerated 210% in 2000 to 2005 (Fig. 2F) to flow 13 to 14 km/year at the calving front, which is the largest speed in Greenland. The ice front retreated about 10 km. The 8-km/year additional frontal speed over the last 30 km must have longitudinally stretched the 1-km-thick ice to thin it by 250 m. The acceleration increased the mass loss from 5 km^3 ice/year in 1996 (*12*) to 36 km^3 ice/year in 2005 (Table 1), which is 6% of Greenland's total accumulation.

Farther south, Helheim Glacier exhibited a positive mass budget in 1996 to 2004 (*12*) but was thinning at low elevation in the 1990s (*2*). In 2000 to 2005, the glacier accelerated 60% and retreated 5 km (Fig. 2G). The 6-km/year increment in speed over 40 km must have thinned the glacier by 75 m. Its mass balance decreased from positive in 1996 to −12 km^3 ice/year in 2005, which is half the glacier annual accumulation.

Even more pronounced changes are taking place in the southeast, where most glaciers have no names (names in Fig. 2I are mostly associated with fjords) and are rarely visited. Snow accumulation is the highest in Greenland, causing high rates of ice discharge per unit area. This region was rapidly thinning up to the ice divide in the 1990s (*1*) and losing 17 km^3 ice/year over 38,000 km^2 in 1996 (*12*). Here, we estimate a 29-km^3/year ice loss over a more comprehensive area of 73,700 km^2 in 1996 (Table 1). The largest 21 glaciers accelerated 28.5% on average between 1996 and 2000 and 57% in 1996 to 2005 (Fig. 2I). Flow acceleration varies substantially among glaciers but remains widespread and systematic. Most glacier fronts retreated several km since 1996. Total loss increased from 48 km^3/year in 2000 to 67 km^3/year in 2005, which is twice the 1996 value.

Few large glaciers drain the south and southwestern tips of Greenland because its ablation area is much broader and less steep than that in the southeast, so glacier ice discharge at the coast is low. Ice was thickening inland and thinning at low elevation in the 1990s (*1*). We have no thickness data and few velocity data for the largest glaciers. Nordbogletscher (area 14), Sermilik (area 15), and Kangiata nunata/Narssap sermia (area 17) have, respectively, balance fluxes of only 1, 6.5, and 6 km^3 ice/year, so potential mass losses from ice dynamics are small. Kangiata nunata sermia sped up by 6% in 1996 to 2000 and 27% in 2000 to 2005, whereas Narssap sermia sped up by 68% and 150% (Fig. 2K). In areas 18 and 19, where ice flows only a few hundred meters per year, we detected a 25% acceleration in 2000 to 2005 (Fig. 2J). This region is unlikely to experience a positive mass balance at present.

Jakobshavn Isbrae underwent a 95% increase in frontal speed in 1996 to 2005 during the progressive breakup of its floating ice tongue (*13*, *14*) (Fig. 2H). In retreat since before the beginning of the century, the glacier was thickening in 1993 to 1998 (*2*) and then thinning (*15*). Its ice flux, deduced from radio echo sounding and seismic data (*16*), was 27 km^3 ice/year in 1996. Ice discharge increased from 24 km^3 ice/year in 1996 to 46 km^3 ice/year in 2005 (Table 1).

Farther north, Kangilerngata and Eqip sermia accelerated by 30% in 2000 to 2005, but the adjacent larger Sermeq avangnardleq and kujatdleq slowed down by 11% (Fig. 2M), so overall losses did not change. Rinks Isbrae (area 23) did not accelerate in 2000 to 2005 (Fig. 2N) but exhibits a negative mass balance. Similarly, Upernavik Isstrøm is 30% out of balance and

Table 1. Mass loss of the Greenland Ice Sheet. A, area of drainage basin; F, ice flux upstream of ice front in 1996 (2000 if 6th column is blank); D, discharge (D = F − surface mass balance between gate and ice front); SMB, surface mass balance over entire drainage; MB, mass balance (MB = SMB − D). Missing values in 6th and 8th columns are replaced by 7th column in totals. Loss of nonsurveyed west glaciers is extrapolated from average loss per unit area for areas 21 to 31. Area 19 is for flux gate only. Total ice sheet mass balance, Total + SMB anomalies = Total (ice dynamics for North + East + West) + deviations in SMB from 1960 to 1990 average (*19*).

Glacier	A (km²)	F (km³/year)	D (km³/year)	SMB (km³/year)	MB 1996 (km³/year)	MB 2000 (km³/year)	MB 2005 (km³/year)
Tracy/Heilprin (32)	10,439	2.4	1.9	1.7	−0.2	−0.2	−1.0
Humboldt (33)	47,370	5.6	3.7	2.4	−1.3	−1.3	
Petermann (1)	73,927	12.2	11.8	11.1	−0.7	−0.7	−0.7
Ryder (2)	29,832	4.3	3.6	4.2	+0.6	+1.0	+0.5
Ostenfeld (3)	11,166	2.2	1.9	1.5	−0.4	−0.4	
Academy/Hagen (4)	32,386	3.7	3.5	3.4	−0.1	+1.1	−0.7
Nioghalvfjerdsbrae (5)	103,314	14.3	13.5	11.5	−2.0	−1.0	−0.1
Zachariae I. (6)	91,780	11.7	9.9	9.4	−0.5	−0.5	−1.7
Storstrømmen (7)	64,662	6.8	0.1	2.4	+2.2	+2.3	+3.3
North	464,876	62.8	50.0	47.6	−2.4	−0.7	−2.3
Daugaard-Jensen (8)	50,150	10.5	10.0	10.0	+0.0	+0.0	+0.0
Kangerdlugssuaq (10)	51,027	27.9	27.8	22.6	−5.2	−5.2	−35.8
Helheim (11)	48,140	26.2	26.3	30.1	+3.8	+3.8	−12.0
Ikertivaq (12)	10,327	10.3	10.1	9.2		−0.9	−3.0
Southeast (13)	63,413	67.4	66.8	37.5	−29.3	−48.3	−67.4
East	223,057	142.3	141.0	109.4	−31.6	−50.6	−118.2
Nordenskiold (19)	62,647	10.7	10.7	14.1		+3.4	−0.7
Jakobshavn I. (20)	92,080	27.0	23.6	30.0	+6.4	−12.5	−16.0
Sermeq kujatdlEq. (21)	25,647	10.9	10.0	6.8		−3.2	−3.2
Kangerdlugssup (22)	7110	2.7	2.4	1.9		−0.5	−0.5
Rinks (23)	30,182	12.1	11.8	10.6		−1.2	−1.2
Upernavik (24)	22,471	8.6	8.1	6.0		−2.1	−2.1
Nunatakavsaup (25)	14,680	4.7	3.8	2.3		−1.5	−1.5
Igdlugdlip (26)	23,802	7.1	6.2	5.4		−0.8	−0.8
Hayes (27)	34,803	10.9	9.9	8.7		−1.2	−1.2
Steenstrup (28)	6,010	1.3	0.8	1.2		+0.4	+0.4
Kong Oscar (29)	21,134	8.5	8.3	6.8	−1.5	−1.5	−1.8
Peary/Docker (30)	12,717	8.5	7.8	4.2		−3.6	−3.6
Gades (31)	2,983	3.3	3.2	1.1		−2.1	−2.1
Nonsurveyed (30-31)	165,084	64.4	59.2	45.0	−14.2	−14.2	−14.4
West	521,350	180.7	165.8	144.1	−21.7	−40.6	−47.0
Total	1,209,280	386	357	301	−56 ± 30	−92 ± 30	−167 ± 40
Total + SMB anomalies					−91 ± 31	−138 ± 31	−224 ± 41

continues a retreat started early in this century (*17*). Mass balance is strongly negative as well for Igdlugdlip and Nunatakavsaup sermia, Steenstrup, Kong Oscar, Peary, Døcker Smith, and Gades glaciers, and probably all glaciers flowing from the high-accumulation northwestern belt. The fastest glacier, Kong Oscar, accelerated by 12% in 1996 to 2000 and none in 2000 to 2005 (Fig. 2O). Overall, flow acceleration north of 70°N is subdued or absent compared to that in the south. The largely negative mass balance of the northwest sector, however, which is consistent with its observed dynamic thinning (*1*, *2*), suggests that the glaciers were already flowing above balance conditions in 1996. Comparison of the 2000 ice-front velocities with those measured in 1957/58 to 1964 in areas 20 to 23 (*18*) shows no detectable change in speed at the 10% level. If ice dynamics is the cause of thinning, glacier acceleration took place before 1957, and the year 2000 glacier losses have prevailed for many decades.

Glacier losses caused by ice dynamics alone are summarized in Table 1 for north, east, and west Greenland. The largest contributions are from southeast and northwest Greenland in 1996 to 2000, with the addition of central east and west in 2000 to 2005 because of the acceleration of only three large glaciers. These estimates do not include glaciers draining from local ice caps, southwest Greenland glaciers, and small eastern glaciers south of Storstrømmen with low levels of ice discharge.

To obtain the total ice sheet loss, we need to combine the calculated losses from ice dynamics in Table 1 with deviations in surface mass balance from the long-term average calculated elsewhere. Climate warming in the last decade has enhanced surface melt and slightly increased snow precipitation to reduce the surface mass balance compared to the 1960 to 1990 average by an estimated 35 km³ ice/year in 1996 and 46 km³ ice/year in 2000 (*19*), which we linearly extrapolate to 57 km³ ice/year in 2005. Total ice sheet loss, combining dynamic losses and deviations from a zero-anomaly surface mass balance, is then 91 ± 31 km³ ice/year in 1996, 138 ± 31 km³ ice/year in 2000, and 224 ± 41 km³ ice/year in 2005.

Greenland's mass loss therefore doubled in the last decade, well beyond error bounds. Its contribution to sea-level rise increased from 0.23 ± 0.08 mm/year in 1996 to 0.57 ± 0.1 mm/year in 2005. Two-thirds of the loss is caused by ice dynamics; the rest is due to enhanced runoff minus accumulation. Ice dynamics therefore dominates the contribution to sea-level rise from the Greenland Ice Sheet.

Glacier acceleration in the east probably resulted from climate warming. Temperature records at Angmassalik (65.6°N, 37.6°E) show a +3°C increase in yearly air temperature from

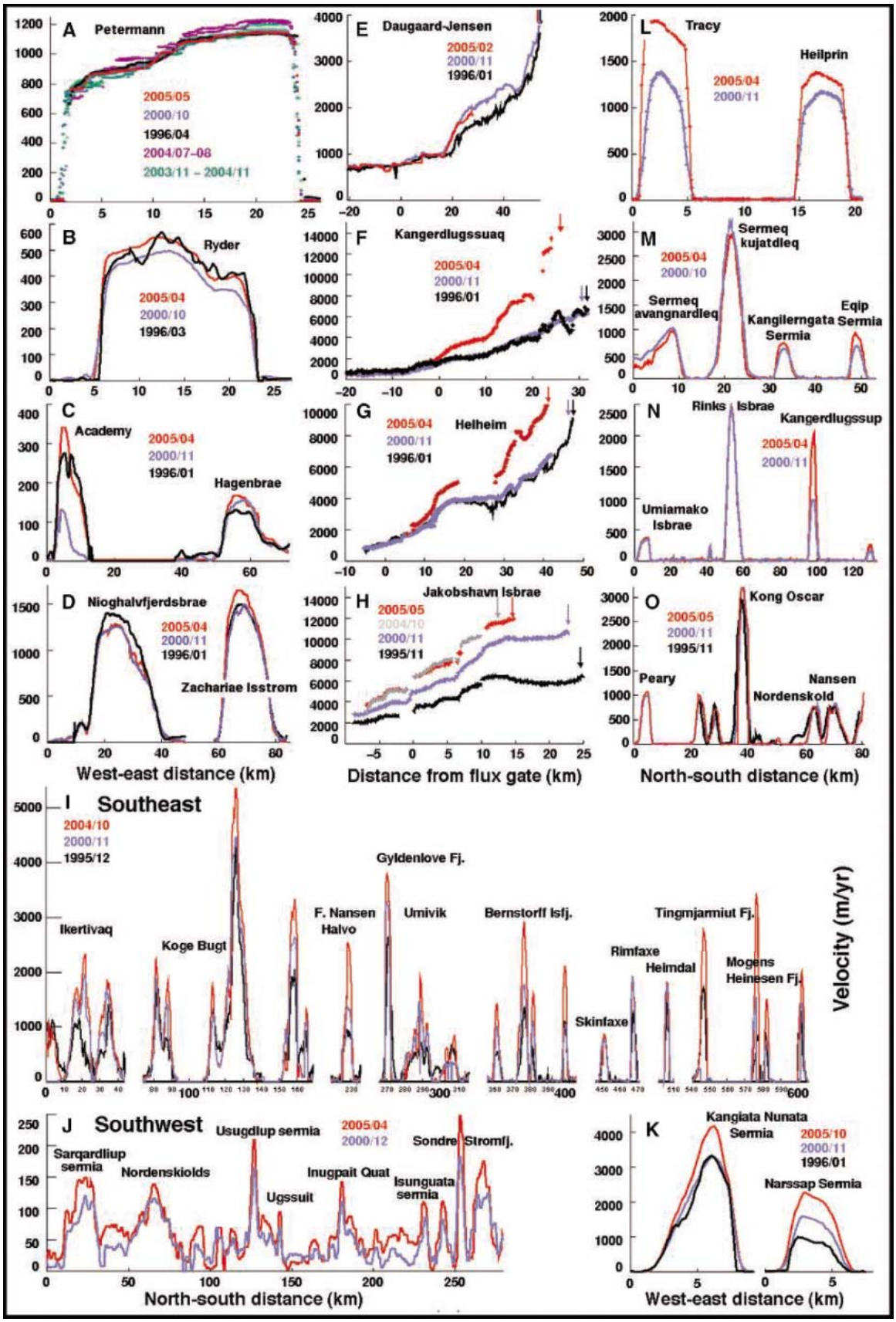

Fig. 2. Ice velocity (in meters per year) for 1996 (black), 2000 (blue), and 2005 (red) of Greenland glaciers versus distance. In (**A**) to (**D**) and (**I**) to (**O**), the selected velocity profiles cross the glaciers within a few km of the ice front, and distance is measured in a direction perpendicular to the glacier flow (west-east or north-south). In (**E**) to (**H**), the selected velocity profiles are in the along-flow direction, at the glacier center line, starting from above the flux gate (left side of the axis, negative distance) toward the ice front (right side of the axis, positive distance). (A) Petermann (1); (B) Ryder (2); (C) Academy/Hagenbrae (4); (D) Nioghalvfjerdsbrae/Zachariae Isstrøm (6, 7); (E) Daugaard-Jensen (10); (F) Kangerdlugssuaq (11); (G) Helheim (12); (H) Jakobshavn Isbrae (20); (I) Southeast (12, 13); (J) Southwest (19); (K) Kangiata nunata/Narssap (17); (L) Tracy, Heilprin (32); (M) Sermeq kujatdleq (21); (N) Rinks Isbrae, Kangerdlugssup (22, 23); (O) Kong Oscar, Peary (29, 30). Arrows in (E) to (H) indicate ice-front positions on different years.

1981–1983 to 2003–2005. The processes that control the timing and magnitude of glacier changes are, however, not completely characterized and understood at present. Glacier accelerations have been related to enhanced surface meltwater production penetrating to the bed to lubricate its motion (*20*), and ice-shelf removal (*13*), ice-front retreat, and glacier ungrounding (*21*, *22*) that reduce resistance to flow. The magnitude of the glacier response to changes in air temperature (surface melting) and ocean temperature (submarine melting at calving faces) also depends on the glacier-bed properties, geometry, and depth below sea level and the characteristics of the subglacial and englacial water-storage systems (*3*, *20*). Current models used to project the contribution to sea level from the Greenland Ice Sheet in a changing climate do not include such physical processes and hence do not account for the effect of glacier dynamics. As such, they only provide lower limits to the potential contribution of Greenland to sea-level rise. If more glaciers accelerate farther north, especially along the west coast, the mass loss from Greenland will continue to increase well above predictions.

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Transitions to Asexuality Result in Excess Amino Acid Substitutions

Susanne Paland and Michael Lynch

Theory predicts that linkage between genetic loci reduces the efficiency of purifying selection. Because of the permanent linkage of all heritable genetic material, asexual lineages may be exceptionally prone to deleterious-mutation accumulation in both nuclear and organelle genes. Here, we show that the ratio of the rate of amino acid to silent substitution (K_a/K_s) in mitochondrial protein-coding genes is higher in obligately asexual lineages than in sexual lineages of the microcrustacean *Daphnia pulex*. Using a phylogeny-based approach to quantify the frequency of mutational-effect classes, we estimate that mitochondrial protein-coding genes in asexual lineages accumulate deleterious amino acid substitutions at four times the rate in sexual lineages. These results support the hypothesis that sexual reproduction plays a prominent role in reducing the mutational burden in populations.

Although sexual reproduction is costly when compared with asexual reproduction (*1*–*3*), it may accelerate the rate of adaptation and inhibit the accumulation of mildly deleterious mutations, because meiotic segregation and recombination facilitate the ability of natural selection to act independently on different genetic loci (*2*–*6*). These effects arise because the stochastic sampling variance associated with the interference between selection on linked loci reduces the genetic effective population size (N_e), which increases the power of random genetic drift (*7*–*9*). As the frequency of recombination is reduced, the fates of mutant alleles become increasingly dependent on the backgrounds in which they originate, and the buildup of repulsion disequilibrium reduces the fitness differential between chromosomes (the Hill-Robertson effect) (*7*), thereby diminishing the efficiency of selection. As a consequence, mildly deleterious mutations may accumulate through several population-genetic mechanisms (*10*–*15*), leading to a long-term decline in fitness. Depending on the distribution of mutational effects, epistatic interactions between consecutive mutations can either slow or accelerate this process (*16*). Although increased rates of nonadaptive evolution have been documented for genomic regions with low levels of recombination and for nonrecombining chromosomes (*17*, *18*), and it is thought that few asexual taxa persist for long periods of time (*19*), it remains to be determined whether mildly deleterious mutations play a critical role in their early demise (*20*).

To evaluate the degree to which sexual reproduction promotes the purging of deleterious mutations, we compared patterns of nucleotide substitution in the 13 protein-coding genes encoded by the mitochondrial genomes (supporting online text) of cyclically parthenogenetic ("sexual") *Daphnia pulex* with those in their obligately parthenogenetic ("asexual") derivatives (table S1). The latter represent independent lineages of recent origin resulting from a dominant sex-limited meiosis suppressor transmitted by male progeny of otherwise asexual lineages (*21*, *22*). We reconstructed a phylogeny by application of a Bayesian method (*23*). Because this species is ancestrally sexual and reversals of asexuality to sexuality are unlikely (and unknown), asexual evolution is represented by sequence changes on branches connecting current asexuals with their most recent sexual ancestors. However, because asexuality may have actually arisen part way down a given asexual branch, the true differences between sexual and asexual sequence evolution reported below will be underestimated, making our test conservative.

The predicted molecular signature of deleterious-mutation accumulation for genes mostly subject to purifying selection ($K_a/K_s < 1$) is an increased rate of evolution at the amino acid level, whereas genes predominantly under

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positive selection ($K_a/K_s > 1$) are expected to show decreased rates of protein evolution (*17*, *24*). The predicted acceleration in mutational decay in asexual lineages extends to the normally nonrecombining mitochondrial genes because the loss of segregation between nuclear and organelle genomes, analogous to the loss of recombination between nuclear loci, subjects such genes to selective interference from the entire nuclear genome. Because animal mitochondrial genomes generally have elevated mutation rates, the power to detect excess rates of mutation accumulation in short-lived lineages is enhanced, and the haploid nature of such genomes simplifies the acquisition of sequence data.

We applied a maximum-likelihood approach (*23*) to the concatenated coding sequences of all 13 mitochondrial protein-coding genes, using hierarchical models to evaluate the validity of alternative assumptions about the mode of evolution in asexual versus sexual lineages and internal versus external branches. Because high rates of adaptive evolution might represent a confounding factor in our analysis, we first evaluated the assumption that the mitochondrial protein-coding genes of *D. pulex* are mostly subject to purifying selection (i.e., $K_a/K_s < 1$). The one-ratio model estimates the average ability of amino acid–altering mutations to accumulate in mitochondrial protein-coding genes by constraining all branches of the phylogeny to have the same K_a/K_s ratio. The resultant overall K_a/K_s ratio of 0.159 (Table 1) reflects a significantly lower amino acid than silent substitution rate. The proportion of strongly deleterious spontaneous amino acid substitutions can then be expressed as $1 - (K_a/K_s) = 0.841$ (*25*), providing a clear indication that purifying selection rejects the majority of amino acid altering mutations in the mitochondrial protein-coding genes of *Daphnia*.

The two-ratio model tests for the presence of mildly deleterious mutations among the pool of observed amino acid substitutions by allowing different K_a/K_s ratios for internal and external branches of the phylogeny. External branches depict the most recent, and internal branches the more distant, evolutionary history of a sample of DNA sequences. If amino acid substitutions are either neutral or strongly deleterious, then K_a will approximate the mutation rate to neutral amino acid substitutions, and the K_a/K_s ratios of external and internal branches should remain constant, assuming that silent substitutions also accumulate at the neutral mutation rate. This hypothesis is rejected because the two-ratio model provides a significantly better fit to the data than the one-ratio model, yielding a K_a/K_s ratio about twice as high for external as for internal branches ($2\Delta\ell = 10.04$; df = 1; $P < 0.005$) (Table 1).

Although this pattern supports the existence of a class of mildly deleterious amino acid substitutions that persist in the short term but are removed by purifying selection in the longer term (*26*), to further evaluate whether purifying rather than positive selection is the predominant form of selection acting on intraspecific amino acid polymorphism in *D. pulex*, we used an extension of the McDonald-Kreitman test of neutral molecular evolution (*27*) to compare the ratio of the numbers of polymorphic amino acid (P_a) and silent (P_s) substitutions among sexual *D. pulex* with the ratio of the numbers of fixed amino acid (D_a) and silent (D_s) substitutions between sexual *D. pulex* and a closely related outgroup species, *D. melanica* (*23*). Predominantly adaptive evolution results in $P_a/P_s < D_a/D_s$, because advantageous amino acid substitutions are fixed relatively rapidly and contribute mainly to interspecific divergence, whereas $P_a/P_s > D_a/D_s$ reflects the signature

Table 1. Maximum-likelihood parameter estimates for the mitochondrial DNA data set and estimates of selective constraint under the three likelihood models.

	*p**	ℓ†	K_a/K_s‡	$1 - K_a/K_s$§
One-ratio model	1	−19,448.09	All branches = 0.159	0.841
Two-ratio model	2	−19,443.07	Internal branches = 0.098	0.902
			External branches = 0.192	0.808
Four-ratio model	4	−19,437.59	Internal sexual branches = 0.091	0.909
			Internal asexual branches = 0.143	0.857
			External sexual branches = 0.135	0.865
			External asexual branches = 0.268	0.732

*Number of free parameters in the likelihood analysis. †Log-likelihood values. ‡Maximum-likelihood estimates of the ratio of the rate of amino acid to silent substitution. §Estimates of selective constraint.

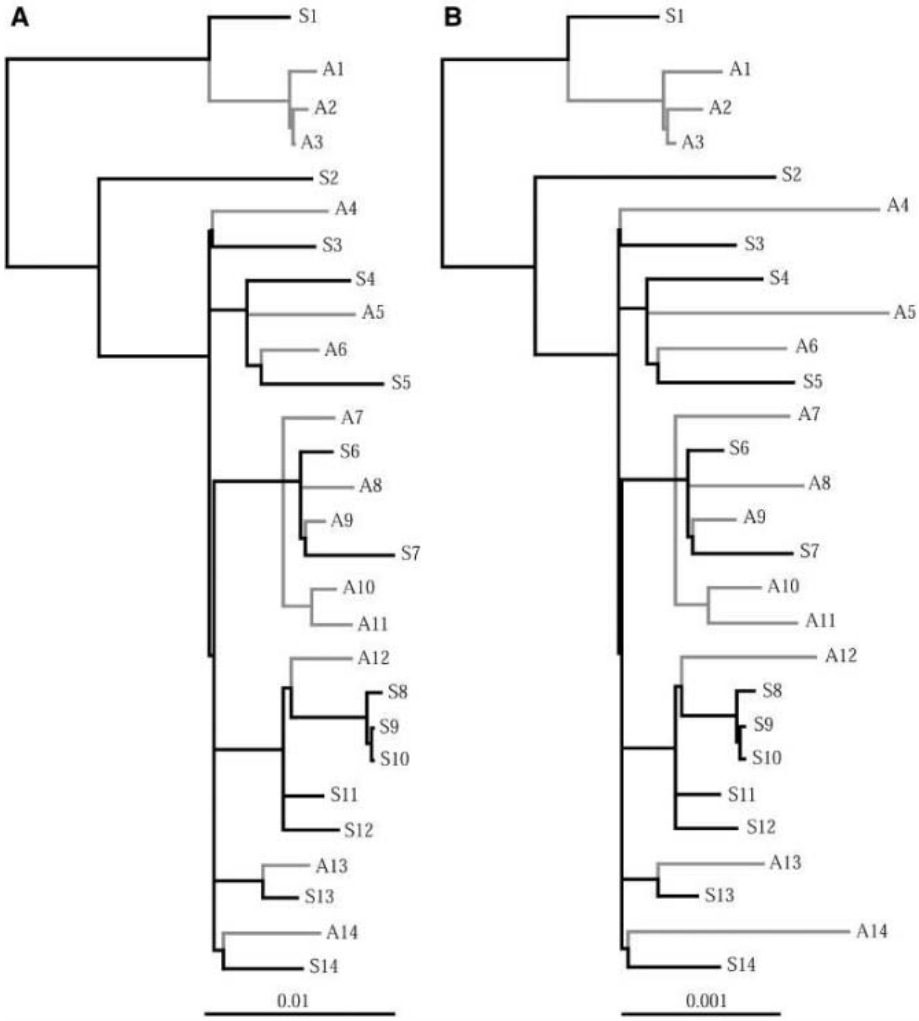

Fig. 1. Phylogenetic trees constructed using maximum-likelihood estimates of the expected number of silent (**A**) and amino acid (**B**) substitutions per site under the four-ratio model. Sexual (S1 to S14) and asexual (A1 to A14) lineages are denoted by black and gray branches, respectively.

of purifying selection against mildly deleterious amino acid substitutions that contribute mainly to intraspecific polymorphism (*28*). P_a/P_s is significantly larger than D_a/D_s ($P_a/P_s = 0.246$; $D_a/D_s = 0.150$; $P = 0.029$), confirming that purifying selection is the predominant form of selection acting on mitochondrial amino acid sequence variation in *D. pulex*. This type of pattern has been consistently reported in other animals (*26*).

A four-ratio model allowing different K_a/K_s ratios for sexual and asexual internal and external *D. pulex* branches fits the data significantly better than the two-ratio model ($2\Delta\ell = 10.96$, df = 2, $P < 0.005$) (Table 1). The estimated K_a/K_s for asexual external branches is twice as high as that for sexual external branches, and K_a/K_s for asexual internal branches is 1.6 times as high as that for sexual internal branches. Because of the recent origin of asexual lineages, there are only three internal asexual branches in our analysis, so the external-branch estimate provides a better indication of the disparity in patterns of sequence evolution between asexuals and sexuals. In any event, it is clear that excess amino acid substitutions occur in asexual lineages. Phylogenetic trees based on maximum-likelihood estimates of the expected number of silent versus amino acid substitutions per site under the four-ratio model have notably different shapes as a result of the elevated accumulation of amino acid substitutions on asexual branches (Fig. 1).

The degree to which the accumulation of deleterious mutations is accelerated in asexual lineages can be quantified by using a phylogeny-based method. This method assumes that (i) silent substitutions accumulate at the neutral rate, here validated by the lack of a significant difference in K_s between sexual and asexual branches (Wilcoxon two-sample test; external branches, $P = 0.505$; internal branches, $P = 0.422$) (see also Fig. 1); (ii) nearly all excess amino acid substitutions in asexual lineages are deleterious; and (iii) the frequency of adaptive amino acid substitutions is negligible. The observed K_a/K_s ratios for sexual and asexual branches of the phylogeny (Table 1) can then be used to estimate the frequencies of (i) strongly deleterious amino acid substitutions, subject to rapid purifying selection in both sexual and asexual populations, as $1 - (K_a/K_s)$ for external asexual branches; (ii) moderately deleterious amino acid substitutions maintained in asexual populations but subject to rapid purifying selection in sexual populations, as the difference between K_a/K_s on external asexual and sexual branches; (iii) mildly deleterious amino acid substitutions segregating in both sexual and asexual populations, as the difference between K_a/K_s on external and internal sexual branches; and (iv) effectively (but not necessarily absolutely) neutral substitutions, as K_a/K_s on internal sexual branches. Of the amino acid altering mutations arising in mitochondrial protein-coding genes of *D. pulex*, we estimate that 73.2% have strongly deleterious effects and are subject to purifying selection irrespective of the population's breeding system, 13.3% have moderately deleterious effects and persist only in asexual populations, 4.4% are mildly deleterious and allowed to persist in the short-term even in sexual populations, and 9.1% are effectively neutral. Thus, the rate of accumulation of deleterious amino acid–altering mutations in asexual lineages, 4.4 + 13.3 = 17.7%, is four times as high as that for sexual lineages (4.4%).

This difference is unlikely to be due mainly to ecological or demographic differences between sexual and asexual populations. Because new asexual lineages of *D. pulex* arise by the backcrossing of asexually produced males to females of the sexual species, not only do members of both lineages necessarily share a common recent biogeographic and ecological history (*22*), but also they contain the same background genomic content relevant to local adaptation. Newly invading asexuals often rapidly replace resident sexual populations, creating lineages with densities of many millions of individuals, so there is no evidence for prolonged demographic bottlenecks. Thus, our results indicate that sexual reproduction enhances the efficiency of purifying selection, supporting the theory that deleterious-mutation accumulation is a leading evolutionary force contributing to the short longevity of asexual lineages.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5763/990/DC1
Materials and Methods
SOM Text
Table S1
References

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Cdx2 Gene Expression and Trophectoderm Lineage Specification in Mouse Embryos

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Controversy exists as to whether individual blastomeres from two-cell-stage mouse embryos have identical developmental properties and fate. We show that the transcription factor Cdx2 is expressed in the nuclei of cells derived from the late-dividing but not the first-dividing blastomere of two-cell embryos and, by lineage tracing and RNA interference knock-down experiments, that this lagging cell is the precursor of trophectoderm. *Cdx2* mRNA is localized toward the vegetal pole of oocytes, reorients after fertilization, and becomes concentrated in the late-dividing, two-cell-stage blastomere. The asymmetrical distribution of *Cdx2* gene products in the oocyte and embryo defines the lineage to trophectoderm.

In most animals, the proper development of the embryo depends on the asymmetrical distribution of maternal transcripts and protein in the egg. In *Drosophila*, gradients of transcription factors are established that provide spatially restricted, *cis*-regulatory control over downstream

zygotic genes (*1*). The *Xenopus* oocyte, although radially symmetrical, also possesses an asymmetrical distribution of mRNA that reflects the animal and vegetal poles of the egg and subsequent patterning during embryogenesis (*2*).

Early mammalian embryos have long been considered to lack the polarity so evident in frogs and insects because of the ease with which individual blastomeres can be manipulated and used to regenerate entire embryos and form chimeras (*3*–*9*). Nonetheless, considerable debate rages about whether the mature mouse oocyte contains factors localized in such a manner that they direct future cell differentiation (*10*–*13*). This argument has recently focused on the individual fates of blastomeres of the two-cell-stage embryo. Whereas some argue that these two cells are equivalent in their developmental properties (*14*–*16*), others have reported that one blastomere contributes predominantly to the embryonic part of the blastocyst [polar trophectoderm and inner cell mass (ICM)] and the other to the abembryonic portion (*17*–*20*). Here, we provide evidence that trophectoderm lineage specification occurs early in development, certainly by the two-cell stage and most likely before the zygote first divides, in agreement with studies indicating the existence of some patterning at the early stages of mouse development (*21*–*23*).

The caudal-type homeodomain transcription factor Cdx2 is required for proper development of trophectoderm in mice (*24*, *25*). We noted that blastomeres of the two-cell mouse embryo could usually be distinguished according to the distribution of Cdx2 protein (Fig. 1). In 71% (37 of 52) of the cases, both blastomeres showed positive fluorescence, but nuclear localization of Cdx2 was observed in only one of them (Fig. 1B and fig. S1). Of the remainder, Cdx2 expression was either more or less confined to one blastomere (10 of 52, 19%) (Fig. 1A) or distributed relatively uniformly between the two blastomeres (5 of 52) without any obvious nuclear localization (*26*). The second polar body was also Cdx2-positive in most two-cell-stage embryos. The nuclear staining for Cdx2 (Fig. 2, G and I) matched the 4′,6′-diamidino-2-phenylindole (DAPI) staining pattern and nuclear architecture, which was often dominated by a large, centrally placed nucleolus. The peripheral ring of fluorescence was not a consequence of the focal plane selected, as is evident from examination of a series of optical sections (Fig. 2, G to I, and fig. S1). Localization of mEomesodermin (mEomes), another transcription factor associated with trophectoderm differentiation (*27*) and used here as a control, did not show any asymmetry in its distribution between blastomeres (Fig. 3 and fig. S2). Figure 2, A to C, shows that oocytes themselves (n = 45) expressed little or no Cdx2 protein, whereas zygotes (n = 56) consistently exhibited clumps of antigen throughout their cytoplasm but no signs of asymmetry in terms of protein distribution (Fig. 2, D to F), which is quite unlike that seen at the two-cell stage (Fig. 2, G to L).

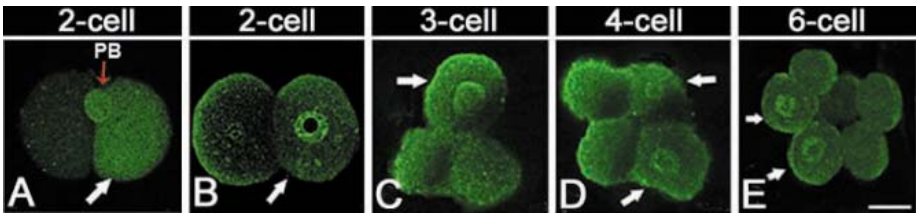

Fig. 1. Localization of Cdx2 (green) in mouse embryos by using an indirect immunofluorescence technique. White arrows indicate blastomeres with perinuclear localization of Cdx2. (**A**) Two-cell embryo with strong cytoplasmic localization of Cdx2 in one blastomere. The red arrow indicates a polar body (PB). (**B**) Typical pattern of Cdx2 expression in a two-cell embryo. (**C** to **E**) Typical pattern of Cdx2 expression in three-, four-, and six-cell embryos. Scale bar indicates 25 μM.

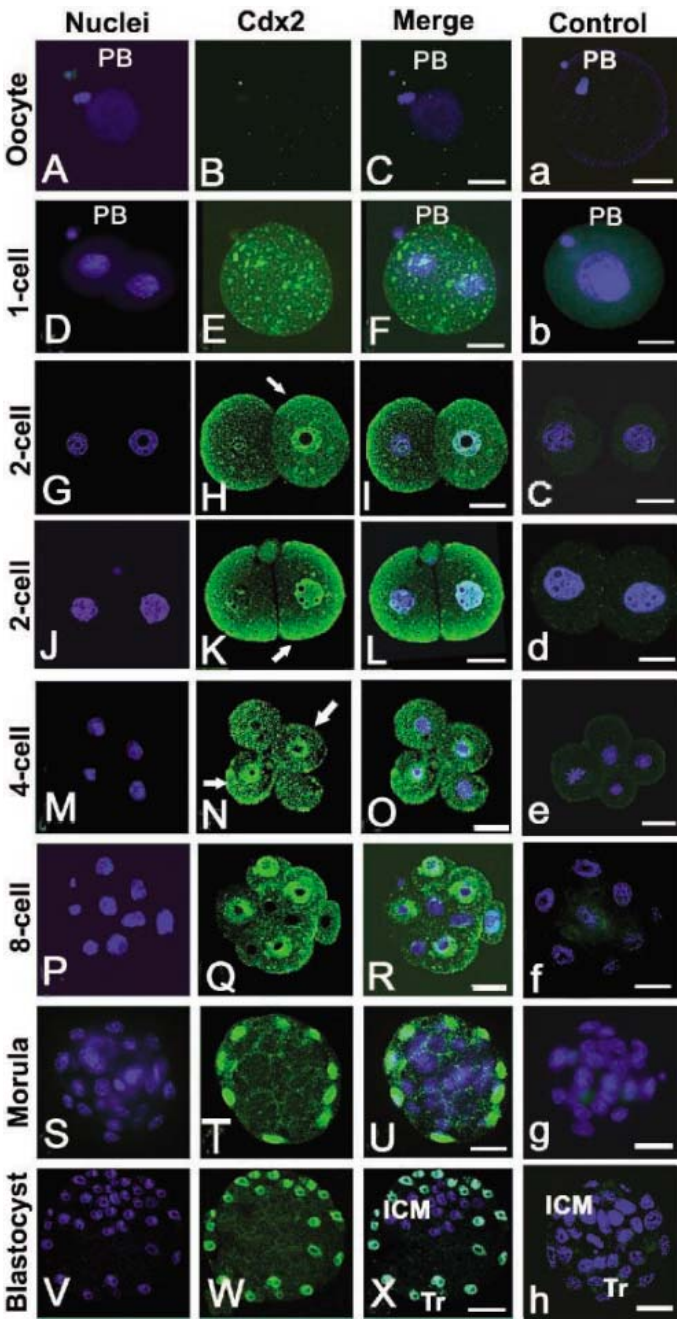

Fig. 2. Cdx2 expression in mouse oocytes and preimplantation stage embryos. (**A** to **C**) Typical unfertilized oocyte with no detectable expression of Cdx2. (**D** to **F**) Zygote with typical punctate staining. (**G** to **I**) Typical two-cell embryo. (**J** to **L**) Optical section from a two-cell embryo showing the intense Cdx2 expression (indicated by white arrows) in the nucleus. (**M** to **R**) Embryos at the four- and eight-cell stages of development with half the blastomeres showing nuclear localization of Cdx2. (**S** to **U**) Typical morula-stage embryo showing nuclear localization of Cdx2 in outer cells. (**V** to **X**) Typical blastocyst with nuclear localization of Cdx2 in trophectoderm. (**a** to **h**) Control blastocyst stained with Cdx2 antibody preadsorbed with recombinant Cdx2 protein. Tr, trophectoderm. Scale bars, 25 μM.

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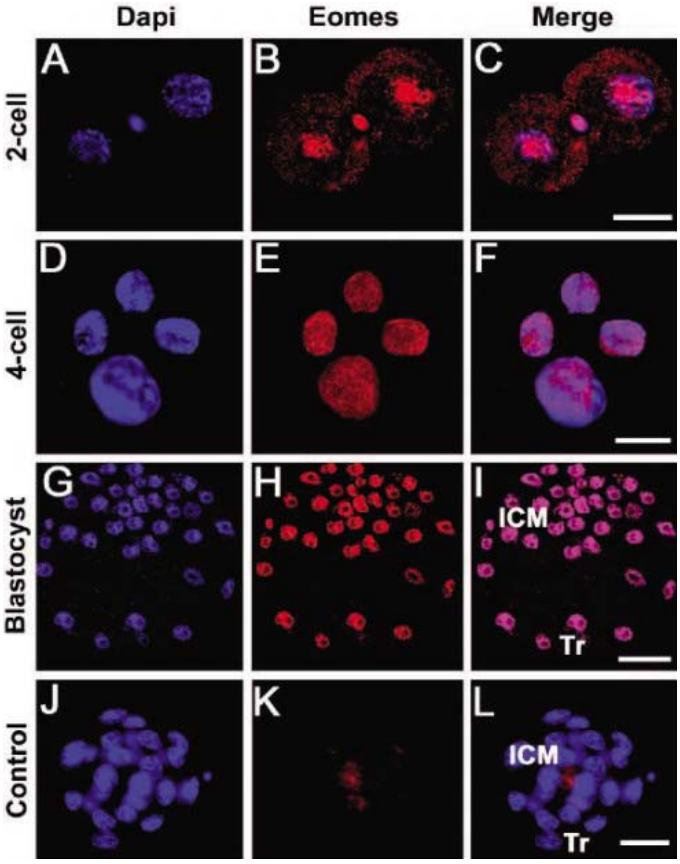

Fig. 3. Distribution of Eomes at two-cell, four-cell, and blastocyst stages of preimplantation development in mouse shown by using immunofluorescence staining of Eomes (red) and nuclei (blue). (**A** to **C**) Two-cell-stage embryos with nuclear and some cytoplasmic staining for Eomes. Scale bars, 25 μM. (**D** to **F**) Four-cell-stage embryos showing nuclear localization of Eomes. (**G** to **I**) Blastocyst showing nuclear localization of Eomes in the trophectoderm (Tr) as well as the ICM cells. (**J** to **L**) Blastocyst stained without the use of the primary antibody for Eomes.

One possibility is that these images of zygotes represent protein either being sorted in some manner or in the process of degradation.

At the three-cell stage, Cdx2 nuclear staining was confined to the largest of the three blastomeres in 88% (53 of 60) of the embryos examined (Fig. 1C). By the four-cell stage, two of the four blastomeres demonstrated nuclear staining (Figs. 1D and 2, M to O, and fig. S3). Therefore, it is the lagging blastomere of the two-cell embryo that expresses Cdx2 in its nucleus. In 47 of 49 embryos examined at the six-cell stage, two blastomeres showed positive Cdx2 nuclear staining, whereas the other four were negative (Fig. 1E). At the eight-cell stage, 52 of 55 embryos exhibited four cells with and four cells without nuclear Cdx2 expression (Fig. 2, P to R, and fig. S4). As expected (*24*), Cdx2 at the late morula stage (~16 cells; $n = 30$) was nuclear and confined to exterior cells of the embryo (Fig. 2, S to U, and fig. S5). At blastocyst ($n = 35$), the protein was localized in nuclei of the trophectoderm and absent from the ICM (Fig. 2, V to X). The asymmetrical staining pattern for Cdx2 shown in Figs. 1 and 2 has been confirmed with a second antibody from a different source. These results suggest that Cdx2 expression patterns can be used to trace the trophectoderm lineage.

To determine whether the late-dividing blastomere from two-cell embryos was the predom-

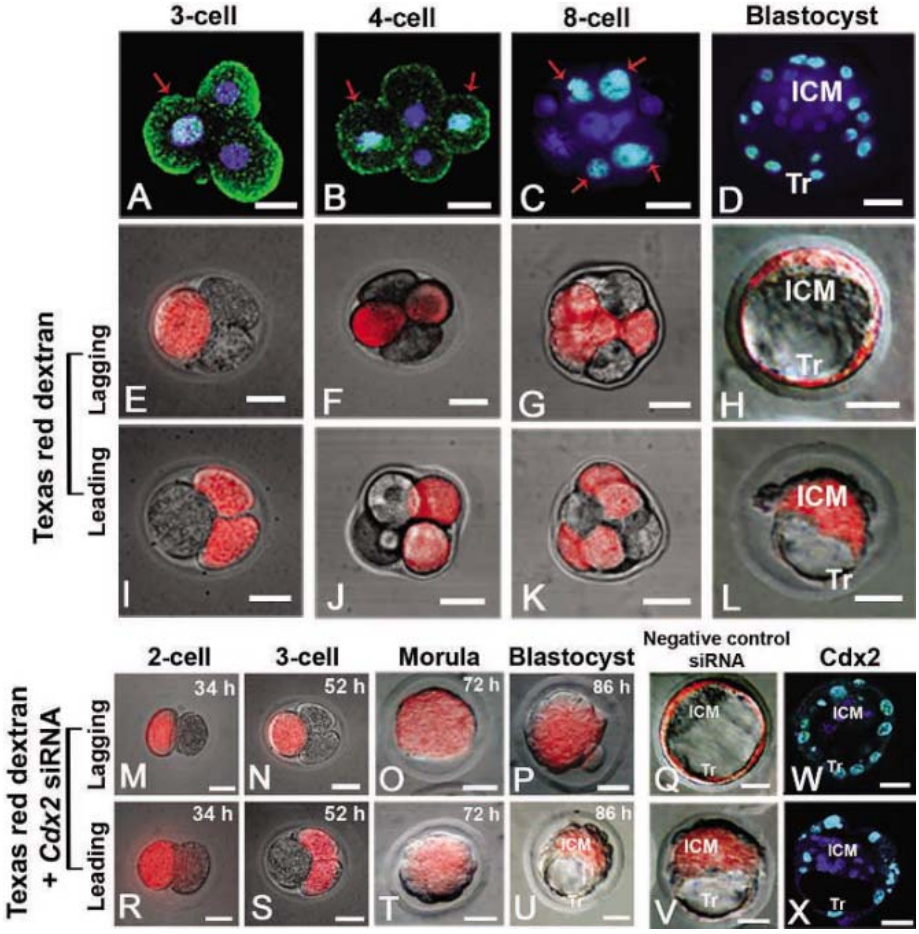

Fig. 4. The lagging two-cell-stage blastomere contributes to the formation of trophectoderm. In (A) to (D), (W), and (X), Cdx2 (green), nuclei (blue), and the merge (cyan) shows Cdx2-positive nuclei. Scale bars, 25 μM. (**A** to **D**) Localization of Cdx2 in the lagging blastomere and its progeny at the three-, four-, eight-cell, and blastocyst stages. (**E** to **H**) Trophectoderm lineage tracing from two-cell-stage embryos injected with TRD in the lagging blastomere (red indicates TRD). (**I** to **L**) ICM lineage tracing from two-cell stage embryos injected with TRD in the leading blastomere. (**M** to **P**) Development of a two-cell-stage embryo coinjected with TRD and siRNA for *Cdx2* in the lagging blastomere (at 34 hours) to an abnormal blastocyst without cavity (at 86 hours). (**R** to **U**) Development of a normal blastocyst from a two-cell-stage embryo coinjected with TRD and *Cdx2* RNAi into the leading blastomere. (**Q** and **V**) Normal blastocyst development after coinjection of negative control RNAi and TRD in the lagging and leading two-cell blastomeres, respectively. (**W**) Blastocyst showing retention of trophectoderm Cdx2 expression after injection of negative control siRNA in the lagging two-cell blastomere. (**X**) Retention of trophectoderm Cdx2 expression in a blastocyst after injection of *Cdx2* RNAi and TRD in the leading two-cell blastomere.

inant lineage precursor of trophectoderm, we injected neutral Texas red–conjugated dextran (TRD) (*28*), molecular weight of 40,000, randomly into blastomeres of two-cell embryos. A majority (37 of 40, 92%) of the embryos survived injection. About half (19 of 37) showed red fluorescence in the lagging blastomere at the three-cell stage (Fig. 3E) and progressed through the four-, eight-cell, and morula stages to the blastocyst (Fig. 3, F to H). All 19 blastocysts that formed showed red fluorescence in trophectoderm. The ICM was generally unlabeled except for the region adjoining the mural trophectoderm. To prove that injection of TRD had not caused this effect, we followed the development of embryos (n = 10) in which the TRD-injected blastomere had divided first. All 10 such embryos progressed normally to blastocyst, and nine showed red fluorescence localized to the ICM (Fig. 3, I to L). Therefore, the lagging blastomere at the two-cell stage contributes mainly to trophectoderm, whereas the leading blastomere is the precursor of the ICM. These observations support the previous conclusions of Piotrowska *et al*. (*17*), except our data indicate that the progeny of the lagging blastomere contribute to the polar as well as the mural part of the trophectoderm. The reason for this discrepancy is not clear but could be due to the method used to mark the blastomeres or, more likely, to mouse strain differences.

Next, we determined whether silencing of *Cdx2* in the lagging blastomere would disrupt blastocyst formation by injecting one of the two-cell-stage blastomeres with TRD along with a predesigned *Cdx2* small interfering RNA (siRNA) molecule. Most (54 of 62) of the embryos survived the injection. At the three-cell stage, we separated the embryos according to whether the labeled blastomere had divided first (Fig. 4, R and S) or last (Fig. 4, M and N) and examined the two groups at the morula (72 hours of development) (Fig. 4, O and T) and blastocyst (86 hours) stages of development (Fig. 4, P and U), as well as at several intermediary stages in a separate experiment (*26*) to track labeled cells. All of the embryos (24 of 24) in which the unlabeled blastomere had been the first to divide either became arrested at the morula stage or gave rise to abnormal structures with no expanded blastocoel cavity (Fig. 4P). In addition, the outer cells of these developmentally compromised embryos failed to express either Cdx2 itself or cytokeratin Endo-A (*29*) (fig. S6, I to L), both markers of trophectoderm. Oct4 was expressed in all but a few outer cells of these embryos that lacked apparent trophectoderm (fig. S6K). In the group of embryos where the RNA interference (RNAi) and TRD-injected blastomere had divided first, 93% (27 of 30) of the embryos developed into normal-appearing blastocysts (Fig. 4U) with label largely confined to the ICM. The expected pattern of Cdx2, cytokeratin Endo-A, and Oct4 localization occurred in these blastocysts (Fig. 4X and fig. S6, B to E). Therefore, injection of RNAi targeted to *Cdx2* in the lagging blastomere leads to a failure of trophectoderm formation.

We next examined the presence of Cdx2 transcripts in oocytes, zygotes, and developing embryos by in situ hybridization. The results were surprising. Of 30 oocytes examined, 25 had *Cdx2* mRNA concentrated in one-half of their cytoplasm (Fig. 5, A to C; for controls, Fig. 5a). In at least 21 of this group of 25 oocytes, the hybridization signal for *Cdx2* mRNA was located on the side of the egg opposite the position of the first polar body, i.e., toward the vegetal pole (fig. S7). The remaining oocytes showed a less pronounced polarity (*26*). We also found that the expression of *Cdx2* mRNA and its localization to one hemisphere of the oocyte in preovulatory oocytes in metaphase II arrest was not evident at metaphase I (fig. S8).

After fertilization, there was a reorientation of the *Cdx2* mRNA relative to the position of the polar bodies from the vegetal pole toward the animal pole, so that *Cdx2* transcripts became concentrated to one side of the axis that bisects the

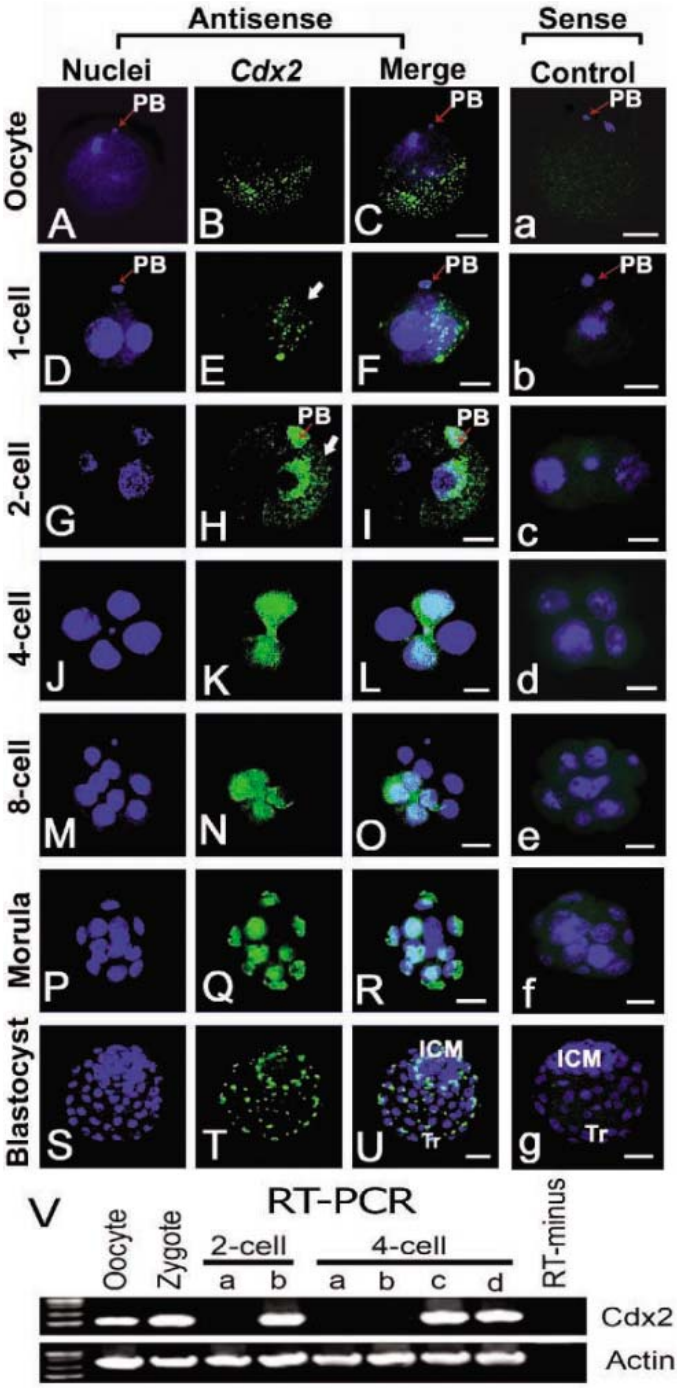

Fig. 5. Distribution of *Cdx2* mRNA in oocytes and at various stages of mouse preimplantation development. *Cdx2* mRNA localization, green; nuclear material, blue. Red arrows indicate PBs. White arrows illustrate the general location of the *Cdx2* signal. Scale bars, 25 μM. (**A** to **C**) *Cdx2* mRNA in unfertilized oocytes. (**D** to **F**) Typical zygote with *Cdx2* mRNA localized to one-half of the cell. (**G** to **O**) Typical embryos at the two-, four-, and eight-cell stages with prominent localization of *Cdx2* mRNA in only half of the blastomeres. (**P** to **R**) Morula-stage embryo with *Cdx2* mRNA localized in the cytoplasm of its outer cells. (**S** to **U**) Blastocyst with *Cdx2* mRNA localized to the trophectoderm. (**a** to **g**) Controls at all stages hybridized to sense probe. (**V**) RT-PCR analysis for *Cdx2* mRNA in oocytes, zygotes, and individual two- and four-cell blastomeres. RNA was extracted from single oocytes, single zygotes, and individual blastomeres from two-cell- and four-cell-stage embryos and amplified as cDNA by RT-PCR. Lane 1, a single ovulated oocyte; lane 2, a single one-cell zygote; lane 3 (2-cell a), one blastomere from a two-cell-stage embryo; and lane 4 (2-cell b), the second blastomere from the same two-cell-stage embryo. Lanes 5 to 8 (four-cell a to d), individual blastomeres from a single two-cell embryo. Lane 9, negative RT control.

animal and vegetal poles. Presumably, this shift reflects the reorganization of cytoskeletal components after the egg is fertilized (*30*). As a consequence, Cdx2 mRNA and presumably other transcripts associated with components of the cytoskeleton (*31*) can then be distributed unequally between the two blastomeres when the zygote divides.

The asymmetry in *Cdx2* mRNA distribution observed in oocytes persisted in embryo stages: one-cell (23 of 32), two-cell (25 of 33), four-cell (26 of 36), and eight-cell (30 of 38) (Fig. 5, D to O), although it remains unclear whether the transcripts are entirely of maternal origin or newly transcribed from the embryonic genome. In most instances, the asymmetry was remarkable, with half the blastomeres showing little evidence for the presence of *Cdx2* transcripts. The relatively homogeneous distribution of *Eomes* mRNA in control oocytes and embryos indicated that the asymmetry of *Cdx2* was not an artifact (fig. S9). At the blastocyst stage, *Cdx2* mRNA was confined to trophectoderm and absent from the ICM (21 of 28) (Fig. 5, S to U). The signal in blastocysts, although outside the nuclei, was generally highly concentrated. We have no explanation for this phenomenon, although it might relate to fixation or permeability artifacts. We confirmed the asymmetric distribution of *Cdx2* transcripts in two- and four-cell embryos by performing reverse transcription polymerase chain reaction (RT-PCR) on RNA from individual blastomeres dissected from embryos (Fig. 5V) and likewise proved that *Cdx2* mRNA was present in metaphase II oocytes. Together, these data show that the localization of *Cdx2* mRNA in the oocyte accurately predicts the lineage of cells destined for trophectoderm.

One puzzle is why $Cdx2^{-/-}$ embryos have been reported to form temporary (*24*) or even complete (*25*) blastocoel cavities, whereas our studies indicate that knock-down of *Cdx2* mRNA in the lagging blastomere of two-cell-stage embryos leads to complete trophectoderm failure. The most likely explanation is that sufficient maternal oocyte *Cdx2* mRNA persists in $Cdx2^{-/-}$ embryos to define the trophectoderm lineage but not to ensure its eventual ability to function.

Our data are at odds with the mainstream view that blastomeres of two-, four- and eight-cell mouse embryos are essentially equivalent (*14*–*16*). Instead, they support the opposing view that individual blastomeres of early embryos can have dissimilar fates (*17*–*23*).

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Supporting Online Material

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X-ray Structure of a Self-Compartmentalizing Sulfur Cycle Metalloenzyme

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Numerous microorganisms oxidize sulfur for energy conservation and contribute to the global biogeochemical sulfur cycle. We have determined the 1.7 angstrom–resolution structure of the sulfur oxygenase reductase from the thermoacidophilic archaeon *Acidianus ambivalens*, which catalyzes an oxygen-dependent disproportionation of elemental sulfur. Twenty-four monomers form a large hollow sphere enclosing a positively charged nanocompartment. Apolar channels provide access for linear sulfur species. A cysteine persulfide and a low-potential mononuclear non-heme iron site ligated by a 2-His-1-carboxylate facial triad in a pocket of each subunit constitute the active sites, accessible from the inside of the sphere. The iron is likely the site of both sulfur oxidation and sulfur reduction.

The microbial oxidation of reduced inorganic sulfur compounds and elemental sulfur to sulfate is one of the major reactions in the global sulfur cycle (*1*, *2*). These reactions provide the energy for the growth of many microorganisms that are essential in biotechnological processes, such as biomining of base and precious metal sulfide ores. Sulfur oxidation also plays an important role in the formation of acid mine drainage, a process that causes serious environmental problems (*3*). In addition, sulfur is among the major energy sources in the light-independent ecosystems of hydrothermal vents, solfataras, and hot springs (*4*), where chemolithoautotrophic microorganisms constitute the basis of the food chains.

Despite much research (*1*, *2*, *5*, *6*), the only example of sulfur oxidation understood at a molecular level is the oxidation of thiosulfate by the Sox complex, the prototype of sulfur compound–oxidizing enzymes isolated from the periplasm of mesophilic and neutrophilic bacteria. Here, both sulfur atoms of the substrate are oxidized to sulfate without formation of free intermediates and with c-type cytochromes as electron acceptors (*5*, *6*).

Much less is known about the oxidation of the virtually insoluble elemental sulfur (5 μg/l H_2O at 25°C) (*7*) and about sulfur-compound oxidation in acidophilic microorganisms, although these are the major players in bioleaching and acid mine drainage formation. Sulfur oxygenase reductases (SOR) are the best known examples of enzymes that oxidize elemental sulfur. They are different from other enzymes because they catalyze a distinctive oxygen-dependent sulfur disproportionation reaction with sulfite, thiosulfate, and hydrogen sulfide as products (Fig. 1C) (*8*, *9*). External cofactors

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or electron carriers are not required. The cytoplasmic SOR has been isolated from three different thermophilic and acidophilic Archaea (fig. S1A) and catalyzes the initial step in the sulfur oxidation pathway in these organisms (*8*–*12*). The *Acidianus ambivalens* SOR is a homomultimeric thermozyme with a temperature range of 50°C to 108°C (*8*, *12*). It contains a mononuclear non-heme iron site with a reduction potential unusually low for this type of iron center (–268 mV) (*12*).

Here, we describe the x-ray structure of the recombinant *A. ambivalens* SOR and assign structural determinants for its reaction mechanism. We propose that a cysteine-bound oligomeric sulfane sulfur chain is the substrate of disproportionation and oxygenation at the iron site and, further, that dissolved and linear sulfur species are the actual substrates and not the regular α-S_8 ring.

The crystal structure of the recombinant SOR was determined by isomorphous replacement with anomalous scattering using crystals soaked with mercury and gold ions (*13*) and refined against 1.7 Å resolution native data (R/R_{free} = 18.3/21.0%) (table S1). The asymmetric unit contains six crystallographically independent SOR monomers. The holoenzyme model (Fig. 1, A and B) was generated by applying the crystallographic four-fold rotation operator of the space group I4. The SOR is a homo-oligomer composed of 24 subunits and forms a hollow sphere with pseudo-432 point-group symmetry and a calculated molecular mass of 871 kD (fig. S2, A and B, and movie S1) (*14*). The sphere has an external diameter of ~150 Å and occupies a volume of 1333 nm^3 (inner cavity included). The dimensions of the SOR oligomer are consistent with the size of the particles previously observed in electron micrographs (*8*, *12*), showing that it represents the biologically active molecule. Striking features of the oligomer's outer surface are six chimney-like protrusions composed of four-helix bundles at the four-fold symmetry axes surrounded by ring-shaped grooves (Fig. 1A and fig. S2D). No continuous channel connects the interior with the surface, because small barriers occlude these "chimneys" (fig. S2, C and D). Solely hydrophobic residues (Phe and Val) contribute to the inner chimney surface (fig. S1A). The enclosed inner compartment has a starlike shape with dimensions of ~71 to 107 Å and a volume of 347 nm^3 (fig. S2D and movie S1). Several small pockets at monomer interfaces and two wider pockets within each individual monomer are connected by narrow channels to the central compartment. Calculated electrostatics of the particle's outer surface show a positive ring surrounding the neutral entrance of the chimneys (fig. S2C). The inner surface is dominated by positive charges, mainly due to the contribution of the Fe^{III} ions (fig. S2D). The SOR's overall architecture resembles that of ferritins, which are smaller proteins displaying an identical point-group symmetry and comprising an inner cavity, which serves as an iron reservoir (*15*).

The monomers have a distorted triangular shape and are symmetrically distributed over the sphere, oriented tangential to the particle (Fig. 1B). The formation of a spherical icosatetramer

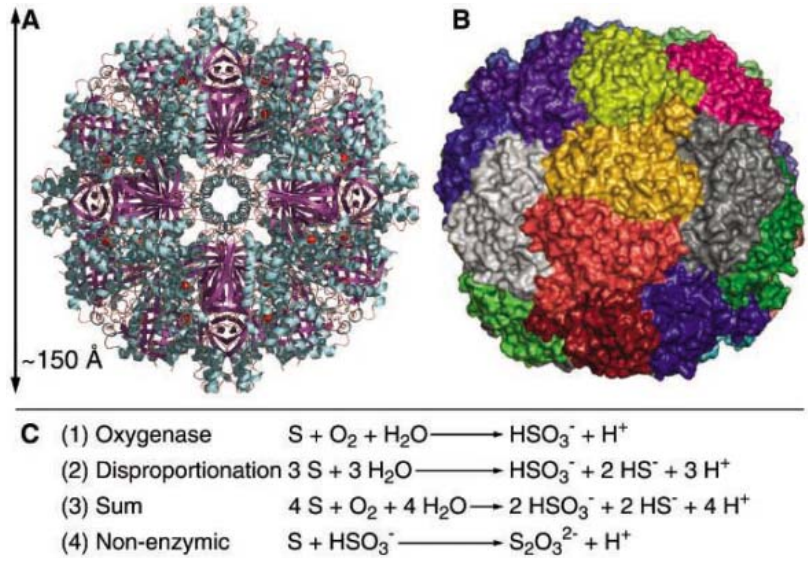

Fig. 1. The SOR holoenzyme. (**A**) Cartoon representation viewed along the crystallographic four-fold axis; α helices are colored cyan, β sheets are purple, and Fe ions are shown as red spheres. Note the protrusions at four-fold pseudosymmetry axes. (**B**) Molecular accessible surface representation with monomers colored differently and viewed along a noncrystallographic two-fold axis. Each monomer has five neighbors. (**C**) The catalytic reaction is an oxygen-dependent sulfur disproportionation with a 1:1 stoichiometry of oxidized and reduced products (Eq. 3) (*8*, *9*). Formally, it can be split into a sulfur oxygenation and a sulfur disproportionation reaction (Eqs. 1 and 2). Thiosulfate is thought to result from a nonenzymic reaction (Eq. 4) under excess S^0.

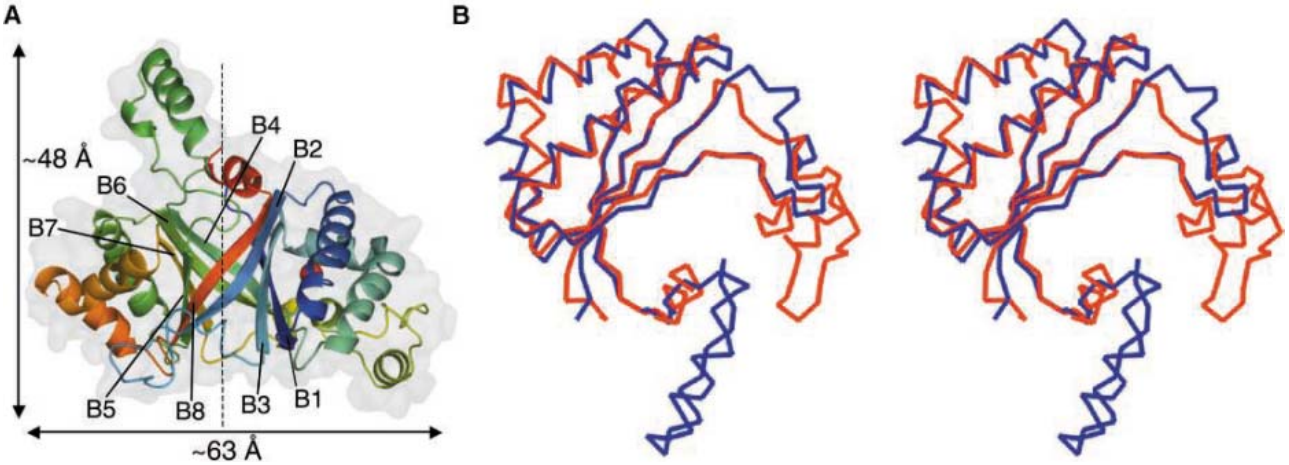

Fig. 2. The SOR monomer. (**A**) Cartoon representation showing secondary structure elements (B, β sheets) colored from blue (N-terminal) to red (C-terminal); red sphere, Fe ion; gray, the monomer's envelope; dashed line, the β barrel's two-fold pseudosymmetry axis approximately parallel to the paper plane. (**B**) Stereo view of the superposition of the two monomer halves, showing their high degree of structural similarity.

implies the formation of distinct intermediate assembly stages. We have postulated a homodimer as the basic building block of the holoenzyme from biochemical data (*12*). Analysis of the intersubunit contacts showed that two monomers share 16% of their total surface area, nearly twice as much as other monomer pairs (table S2). These monomers likely form the homodimeric building blocks (fig. S3A).

The SOR monomer is an αβ protein, formed by an internal β barrel partially surrounded by α helices (Fig. 2A and fig. S3B). The β barrel is formed by eight antiparallel β strands showing remarkable pseudosymmetry of two sets of four strands each (residues 2 to 154 and 155 to 308, respectively) (fig. S1B), related by a two-fold rotation through the barrel axis (Fig. 2A). Each half is reminiscent of the ferredoxin $(\beta\alpha\beta)_2$ topology (fig. S3, C and D) (*16*). The barrel differs from any of the three classical antiparallel β barrels (*17*), but it was recently found in two monooxygenases (Protein Data Bank entries 1lq9 and 1sqe) (*18*, *19*) and in two proteins of unknown function (1t0t and 1vdh) (*20*). Superimposing the two halves reveals a 70% overlap between C_α's to a 1.75 Å cutoff and a root mean square deviation of 1.6 Å (Fig. 2B), despite low sequence similarity in the structure-guided alignment (fig. S1B). The presence of two similar domains in one protein was first described for bovine liver rhodanese (*21*) and was later found in many protein structures (*22*), most of which show low sequence similarity between the domains. These likely result from a gene duplication and fusion event of an ancestor protein.

Three conserved cysteines (Cys31, Cys101, and Cys104 in *A. ambivalens* numbering) (fig. S1A) and a mononuclear nonheme iron site are important for catalysis (*12*, *23*, *24*). These components are located in close vicinity along a cavity within each monomer, constituting the enzyme's catalytic pocket (Fig. 3B). The minimal Fe-Fe distances are ~37 Å, which makes electronic interactions between single centers unlikely (movie S1), although possible cooperative effects cannot be excluded. The 24 catalytic pockets are deeply buried in the interior of each subunit. They are accessible exclusively from the interior of the sphere by a narrow channel from the central compartment (Fig. 3B and fig. S4A). Two methionines, Met296 and Met297 (fig. S1A) are located at the entrance of the pockets. They show flexible side chains, as judged from their alternating conformations and atomic displacement parameters, which might ease substrate access.

Each mononuclear non-heme iron center is located at the distal end of the catalytic pocket. The iron is ligated to His86, His90, and Glu114 (bidentate) (Fig. 3A). Two solvent water molecules, Wat1 and Wat2, complete a distorted octahedral coordination sphere (fig. S4B). The nature and spatial distribution of the protein ligands characterize them as a variation of the

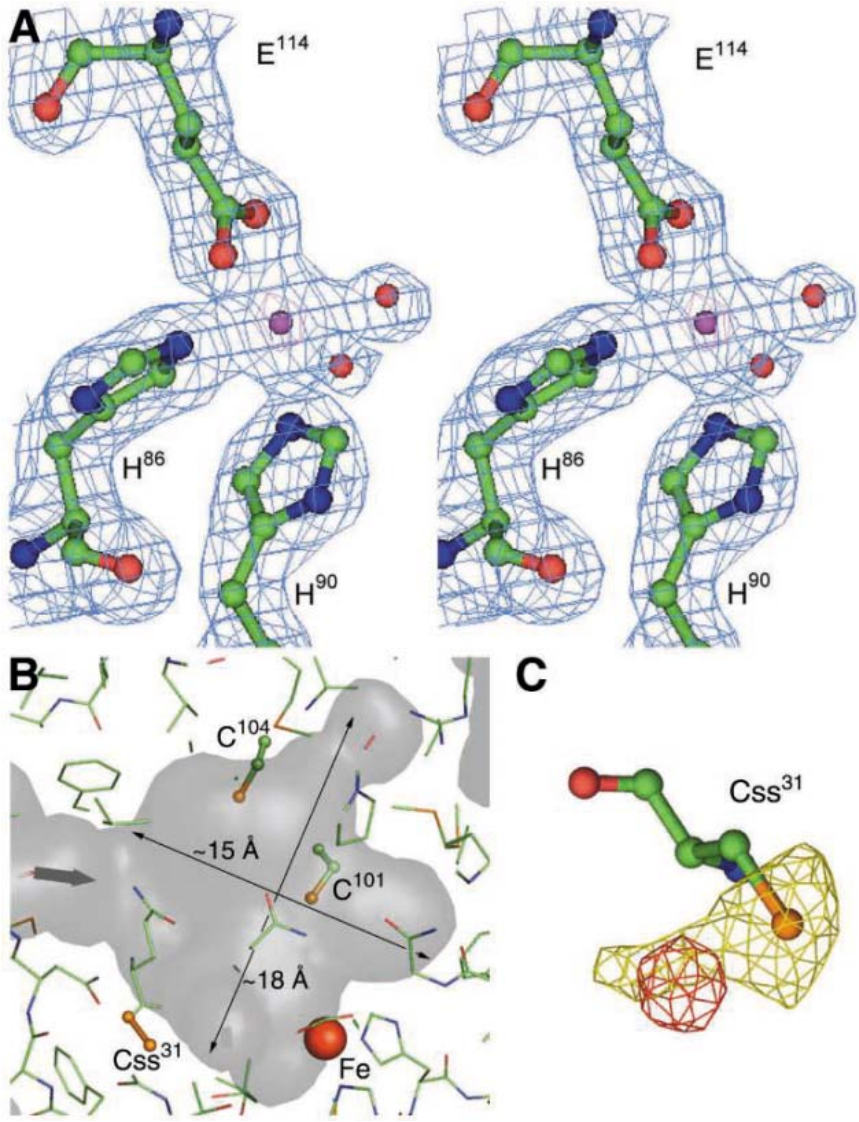

Fig. 3. The catalytic pocket containing the conserved cysteines and the iron. (**A**) Stereo view of the mononuclear nonheme iron center with Fe ligands, electron density contoured at 1 σ; purple sphere, Fe ion; red, waters; ball-and-stick, protein ligands. (**B**) Cavity surface representation of the catalytic pocket with Cys and Fe highlighted; gray arrow, cavity entrance. (**C**) Identification of an additional sulfur atom at Cys31. The position of the additional atom is indicated by the red mesh blob in a six-fold averaged difference Fourier (m|Fo| − D|Fc|) map contoured at 5 σ. The sulfur nature of the additional atom was confirmed by the six-fold averaged anomalous difference map in yellow mesh, calculated using the iron κ-edge data contoured at 3 σ.

2-His-1-carboxylate facial triad motif (*25*). The Fe ion is typically coordinated by two His and one Asp or Glu residue, constituting one face of an octahedron, with the opposite face of the coordination sphere free for the addition of exogenous ligands. Numerous metalloenzymes with nonrelated activities use this structural motif for iron binding and catalysis. Although the carboxylate ligand is monodentate in most cases, there are also examples for bidentate liganding (*25*–*27*). The protein ligands are conserved in all SOR sequences known to date (fig. S1A), as is the sequence motif H-x_3-H-x_{23}-E. Functionally, the structural similarity of the metal center with 2-His-1-carboxylate facial triad enzymes points to a mechanistic analogy, with the SOR iron center being the probable site for oxygen binding and activation. The low reduction potential, compared with other 2-His-1-carboxylate facial triad enzymes (*25*), may result from H-bonding around the Fe site. The presence of a strictly conserved acidic residue (Glu87) may also contribute to an increased stabilization, making Fe a weaker oxidant.

We and others have shown by site-directed mutagenesis that only one of the three conserved cysteine residues is essential in catalysis (Cys31) (*23*, *24*). All cysteines are located in the active site pocket at distances between 7.8 and 12.4 Å to the iron (Fig. 3B). Cys101 and

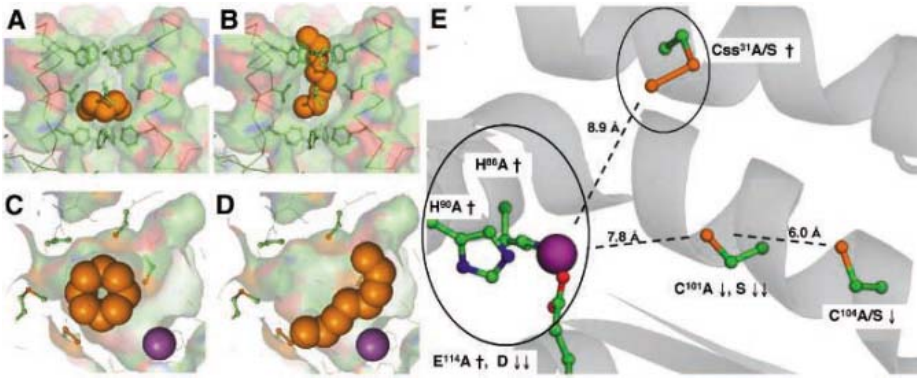

Fig. 4. Substrate access and essential residues for enzyme activity. (**A** and **B**) In silico modeling of α-S_8 sulfur and linear polysulfide, respectively, represented by Van der Waals spheres into the four-helix bundle chimneys, which lead to the interior of the sphere; gatekeeping residues in ball-and-stick representation. (**C** and **D**) α-S_8 sulfur and linear polysulfide modeled as putative substrates at the catalytic pocket, with Fe represented as a purple sphere and catalytic cysteines in ball-and-stick representation; inner surface of catalytic pocket in transparent color. (**E**) Effect of mutants on SOR activity (*23*). The core active site composed of the Fe site and the persulfide-modified Css31 is highlighted within ellipsoids. †, zero activity; ↓, reduced activity; ↓↓, strongly reduced activity.

Cys104 reside in the same helix (Fig. 4E), with their sulfur atoms 6.0 Å apart, too distant to allow disulfide bridge formation even when assuming alternate side-chain conformations. Consequently, no second redox-active site is seen in the SOR, which could assist in performing the rather complex sulfur disproportionation chemistry. The final difference Fourier maps consistently revealed an electron density blob at ~3 to 5 σ at 2.2 Å from Sγ of the essential Cys31 in all six monomers of the asymmetric unit, indicating the presence of an additional atom (Fig. 3C). Averaged anomalous difference Fourier maps using the iron κ-edge wavelength data also showed anomalous scattering at that site, and Cys31 was refined as cysteine persulfide (Css). The terminal sulfane S_δ is oriented toward the iron site, at a distance of 8.9 Å (Fig. 4E). Sulfane sulfur atoms are reactive labile species (*28*), which are generated in the catalytic cycles of various enzymes acting as sulfur transferases in the biosynthesis of sulfur-containing biomolecules (*21*, *29*), but also in enzymes of the sulfur cycle, such as the oxidative thiosulfate transferase from the Sox complex of bacteria (SoxAX) (*30*, *31*) and the polysulfide-binding Sud protein from *Wolinella succinogenes* (*32*). The persulfurated cysteine is the covalent binding site of the substrate in all of these enzymes, indicating that this is also the function of Css31 in SOR, in accordance with mutagenesis studies (*23*, *24*).

The presence of the Css modification in the recombinant SOR crystal structure is remarkable, because the enzyme has not undergone catalytic turnover, and indicates that persulfuration is established before catalysis. There is accumulating evidence that Css are indeed catalytically active species and not intermediates. The recombinant SoxAX protein was described as persulfurated in the absence of its substrate thiosulfate (*31*). This was also observed in a sulfur transferase from *Azotobacter vinelandii* (*29*). Formation and use of Css may thus be a widespread mechanism in inorganic sulfur biochemistry.

To date, five other SOR proteins or genes are known showing 39% to 88% sequence identity with *A. ambivalens* SOR (fig. S1A), and they likely have a similar three-dimensional fold. Highly conserved regions can be identified when plotting the 77 amino acids onto the monomer structure, which are identical in the available sequences (25% of the *A. ambivalens* SOR) (fig. S3E). Notably, 19 out of 23 (83%) residues in an 8 Å radius around the Fe ion are conserved in all SOR sequences, highlighting the functional relevance of the iron site and its primary and secondary ligand sphere. In addition, 21 out of 38 residues (55%) lining the catalytic pocket are conserved, showing the conservation of functionally important residues during evolution and corroborating our identification of the catalytic pocket.

The architecture of the SOR suggests the enzyme's mode of action. Because the 24 active sites are not directly accessible from the exterior, sulfur must enter the sphere before catalysis. Thus, the SOR creates a reaction chamber separated from the cytoplasmic environment. The six chimney-like protrusions are the likely entry routes. These are characterized by a hydrophobic, apolar inner surface (Fig. 4, A and B). Functionally, they can be interpreted as filters for the selection of the correct substrate. The substrate is then delivered to one of the 24 active sites. It has not been determined whether the substrate of the SOR is cyclic α-S_8 or a linear, more soluble sulfur species, such as a polysulfide. In silico modeling showed that α-S_8 is too bulky to travel across the accessing chimney-like pores or to cross the entrance to the active-site pocket without considerable structural rearrangements, whereas linear polysulfides could more easily pass, which indicates that sulfur is linearized (Fig. 4, A to D, and movie S2). In addition, the positive charge of the inner surface might favor the negatively charged anions over the hydrophobic elemental sulfur. After entering the active-site pocket, the substrate is covalently bound to Css31, which is probably in the negatively charged persulfide state. Upon binding, the substrate gets aligned close to the Fe site (movie S2), which, in mechanistic analogy to other 2-His-1-carboxylate facial triad enzymes, probably results in the displacement of one or both water ligands, leading to a coordinatively unsaturated pentacoordinated Fe site that would be poised to bind dioxygen (*25*, *33*). At that stage, the center is probably in the Fe^{II} state, eventually by a one-electron transfer from the cysteine-bound polysulfide. This would also allow oxygen binding to give a peroxo species as the first intermediate. Interestingly, the low reduction potential of the iron site (E_m = –268 mV) (*12*) is in the range of the H_2S/S^0 couple (E_0' = –270 mV) (*34*), suggesting that this is also the site of sulfur reduction. Because the two other cysteine residues are not essential for catalysis, as shown by site-directed mutagenesis (*23*), Css31 and the iron constitute the enzyme's core active site (Fig. 4E). The fate of the reaction products and their pathway upon leaving the SOR sphere has not been resolved.

The structural similarity between the SOR and ferritins and the presence of related active sites in which the confined substrates react with molecular oxygen suggest that these proteins are an example of structural and, to some extent, functional convergent evolution. In addition, our results show how prokaryotes circumvent the lack of compartmentation within their cytoplasm by generating proteinaceous nanocompartments through the oligomerization of proteins.

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35. The authors wish to thank F. Pfeifer (Darmstadt), M. Teixeira and M. A. Carrondo (both Oeiras) for encouragement and support, T. M. Bandeiras for valuable experimental assistance, and the staff at European Synchrotron Radiation Facility Grenoble (France) and European Molecular Biology Laboratory, Outstation Hamburg (Germany). This work was supported by a grant from the Deutsche Forschungsgemeinschaft (to A.K., Kl885/3-3) and by a Deutscher Akademischer Austausch Dienst (Germany) and Gabinete de Relações Internacionais da Ciência e do Ensino Superior (Ministério da Ciência, Inovação e Ensino Superior, Portugal) collaborative travel grant (to A.K. and C.M.G.). T.U. was awarded a Marie Curie fellowship for the structure determination at Instituto Tecnologia Química e Biológica (Oeiras). The atomic coordinates of the SOR have been deposited in the Protein Data Bank at www.rcsb.org (accession code 2CB2).

Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5763/996/DC1
Materials and Methods
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A Keystone Mutualism Drives Pattern in a Power Function

John Vandermeer[1,2]* and Ivette Perfecto[2]

Data that can be described by a power function are ubiquitous in nature. Although there is consensus that such data frequently emerge generally from nonlinear complex systems, a variety of specific mechanisms may be responsible for creating the pattern in particular cases. Here, we report on the distribution of a scale insect (*Coccus viridis*) that is a common agricultural pest. Its distribution in an organic coffee farm in southern Mexico generally follows a power function, but there are subtle deviations from that function. We offer a biological explanation for both adherence to the power functions and associated deviations, along with supporting evidence.

The distribution of clusters of organisms, much like other patterns in nature (*1*, *2*), is frequently characterized by a power function (*3*). For a variety of distinct dynamic scenarios, when cast in a spatial framework, biological interactions of several distinct types are capable of generating spatial pattern characterized by power law scaling (*4*). This pattern is usually interpreted as a signal of self-organization resulting from spatial extension. In most ecological examples, clusters are formed through various biological interactions manifest in a spatial context, such as local depletion of resources (*5*), local disturbance regimes (*6*) or predator-prey interactions (*7*). That clusters form in the first place is an interesting aspect of these theories, but the evidence that the distribution of individuals within those clusters approximates a power law is the most intriguing aspect of self-organization (*2*). Any spatial process that combines a local spread (for example, local production of a prey population) with a regional control (for example, a searching predator that ranges widely) will likely generate clusters (*8*). A similar mechanistic statement is more elusive for the power function nature of those clusters.

For the green coffee scale (*C. viridis*), the formation of clusters is of little interest because each "cluster" is a population of scale insects on a coffee bush. However, the ecology of this organism offers a potential explanation of both the evident power law and subtle deviations from it at high and low population sizes.

The green coffee scale is a common herbivore, frequently noted as a pest in greenhouses and a known pest of coffee (*9*, *10*). Although normally maintained by natural enemies below critical damage thresholds, it can sometimes reach pest status (*11*). It is tended by a variety of ant species in a classic mutualistic form: The homopteron supplies honeydew to the ant, and the ant protects the homopteron from predators (*12*). *Azteca instabilis*, a mutualist of *C. viridis* in southern Mexico (*13*), is a tree-nesting species that occurs in obvious spatial clusters (*14*) in the shade trees of coffee farms. Those coffee bushes that are near a tree occupied by *A. instabilis* are frequently sites of large concentrations of *C. viridis*. At least two species of encyrtid wasps are parasitoids on *C. viridis* and the coccinelid beetle *Azya orbigera* is a voracious predator. Direct observations and experimental results indicate that the *A. instabilis* ants are efficient protectors of the scale insects in the face of these natural enemies (*15*), and casual observations leave little doubt that the ants collect honeydew from the scales.

To investigate the spatial pattern of *C. viridis*, we set up a 45-ha plot on an organic coffee farm in southwestern Mexico (*16*), identified each shade tree therein, and assessed whether or not it contained an *A. instabilis* colony (*13*). Of 10,597 trees located, 276 contained *A. instabilis* colonies. We systematically chose five locations surrounding such a colony and four locations that were clearly outside of the influence of any such colony (*17*). In each of the nine sites, we determined the scale abundance (*17*) on approximately 50 to 100 coffee plants, for a total of 678 coffee plants surveyed.

The frequency distribution of scale insect numbers per tree is shown in Fig. 1. A power function is clearly suggested by the approximately linear nature of the points. However, subtle deviations from the power function at both high and low scale densities are also evident by casual observation. Because of the deviations at both high and low densities, we

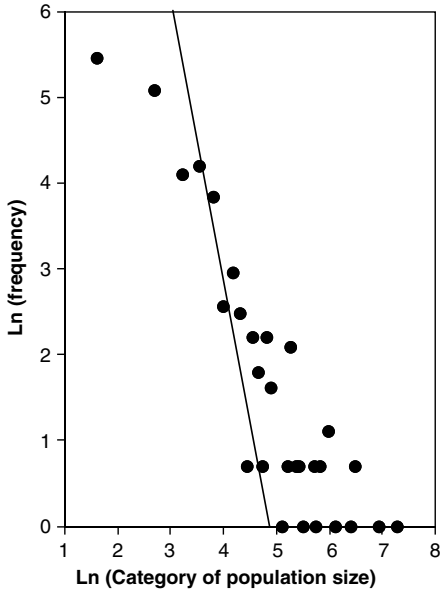

Fig. 1. Log-log plot of population size versus frequency of size of cluster. The line is a regression based on the data points located between 3.5 and 4.5 on the abscissa (*17*). Slope of power function is –2.72.

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considered points in the intermediate range (between log category size 3.5 and 4.5) as a basis for casting a regression line (Fig. 1) (*17*).

Underlying biological processes may account for both the power function and its deviation. Assume that each propagule of *C. viridis* encounters coffee bushes randomly. That propagule will begin population growth that is effectively exponential, at least for a time. Thus, if the exponential growth parameter is normally distributed, the limiting distribution of scales ought to be lognormal. Consequently, we expect a lognormal distribution as the neutral base, which translates directly into a power function. Although this is the expectation at the limit, the approach to that power law may be extremely slow, depending on the mean value of the intrinsic rate of natural increase and its variability. Moreover, the system is substantially complicated by its existence in an extended space. When the exponential process, operative independently at the level of each individual coffee bush, is augmented by random additions of individuals resulting from the spatial extension of the system, the approach to the power-law scaling may be significantly accelerated. The general pattern to be expected can be seen in a simple simulation (*18*), as shown in Fig. 2. Notably, as the overall distribution approaches a lognormal (Fig. 2A), the power function emerges (Fig. 2B). Furthermore, it is the apparent disappearance of the effect of space (i.e., when the late arrivals of populations at bushes are swamped by the population growth on those bushes) that represents a critical state, that is, the state of the pure power function without the deviations (e.g., after eight iterations in Fig. 2). That is, once all or almost all bushes are occupied by at least a single scale insect, the overall dynamics rapidly become dominated by the distributed exponentials with the emerging power law. Before that time, many bushes will have zero or very low occupancies, which maintains an extended tail on the lognormal distribution. Thus, the point of "criticality" is when the random process thought to represent space in this model reaches the critical point of filling all coffee bushes with at least one scale insect. The general idea of criticality and the self-organization that goes along with it (*3*) is thus repeated in this system, although here we are dealing with a second order of critical state, which is to say, the disappearance of the space effects.

Evidence that this dynamic is operative in the system under study is gained from an examination of the frequency distribution of the logs of scale numbers (Fig. 3) compared with the lognormal expected from the underlying biology (local reproduction on a coffee bush), shown by an approximate normal curve superimposed on the data in Fig. 3. It is qualitatively clear from Fig. 3 that there is a strong deviation from the lognormal at the low population size category, much as observed in simulations before the moment of criticality (compare Fig. 3 with Fig. 2A). We thus conclude that the system is near its critical point, in which the random allocation of scales to bushes is almost complete.

However, there is also a less obvious, but equally important deviation from the lognormal distribution at the high end of the spectrum. This is the same deviation that can be seen in the log-log graph presented in Fig. 1. We hypothesize that this high cluster deviation is a consequence of the key mutualism operative in the system. When the coffee bush is near to an ant colony, the inherent limitation on the population, probably due to predation and parasitism, is reduced, and the local population is released to reach much higher values.

Support for this hypothesis is strong if we eliminate those sampling sites in which an *A. instabilis* colony is resident. Plotting only the antless sites from Fig. 1, we obtain the points displayed as solid circles in Fig. 4, which is evidently a linear function with no obvious deviation at high cluster sizes. We take this power law to be the underlying power law that derives from independent populations growing exponentially and randomly dispersing in space. The key mutualism acts to generate a deviation at high cluster sizes from the basic pattern, given by the dotted line in Fig. 4 (with the same slope as the ant-free distribution but with a higher y intercept corresponding to the higher numbers of total scales in the pooled samples).

In sum, the basic power law that *C. viridis* seems to follow is a product of independent populations growing exponentially, giving rise to a basically lognormal distribution, but organized in space such that extremely small populations are common as a consequence of a time delay in the dispersal of individuals to new bushes. The large-cluster deviation from this basic law is caused by the key mutualism between *C. viridis* and *A. instabilis*. Thus, both the power law and the key deviation from the power law are understandable in terms of simple biological processes.

Interpretation of the power law in this particular case is of interest in its own right and represents one of the few cases where it is easy to recognize biological phenomena that can account for the pattern. However, the result may also have practical applications. In the case of a potential pest species, it is always necessary to be able to project the population into the future to determine when control activities may be necessary [the so-called action

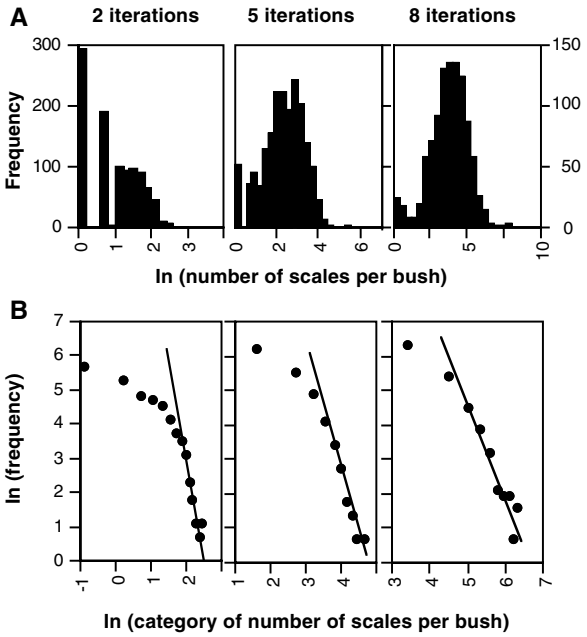

Fig. 2. Results of simulations of the simple exponential model coupled with random dispersal (*18*). (**A**) Frequency diagrams of the log of number of scales per bush for three different iterations. (**B**) Log-log plot of frequency versus category of number of scales per bush, showing how the system approaches a classical power function in time.

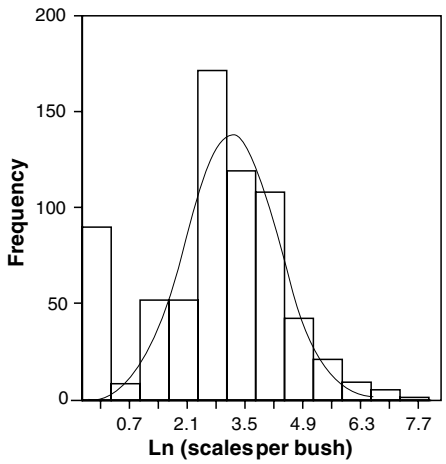

Fig. 3. Frequency diagram of the natural logs of population densities per coffee bush with an approximate normal distribution imposed. The obvious deviation at low cluster sizes is similar to that observed in the simulations (Fig. 2A).

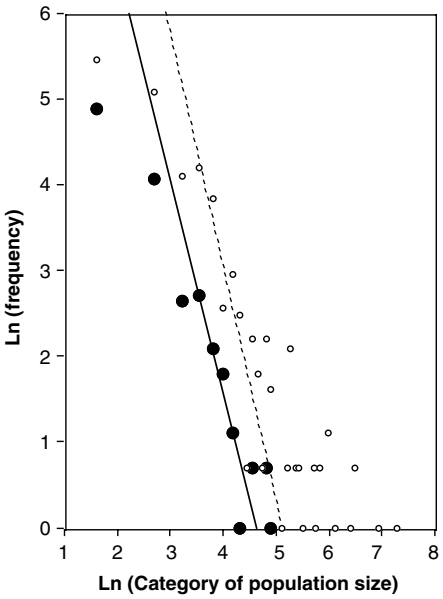

Fig. 4. Log-log plot of population size versus frequency of population sizes for sites away from the influence of an *A. instabilis* colony (solid circles), and all data (small open circles repeat the data of Fig. 1). Slope is −2.48, fitted to the points located between 3.5 and 4.5 on the abscissa (*17*). The evident deviations from the power function at high population densities (small open circles) are not present in this data sample.

threshold (*19*)]. In a spatial context, if all bushes are occupied by scales and density dependence can be ignored, projecting the overall population into the future is a simple matter of applying an exponential function to the total scale abundance. However, if that critical point where all bushes are occupied has not been reached, applying the simple exponential law will necessarily underestimate future population sizes, because the actual spatial dynamic will include newly occupied bushes in the future. Consequently, determining whether the system is at its critical value, at which point the application of the simple exponential would indeed be appropriate, has obvious practical importance. Thus, the degree to which spatial data adhere to a power function can be taken as an indication of the legitimacy of applying an exponential rule to population projections.

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16. The site is located at Finca Irlanda, a 300-ha organic coffee farm in the Soconusco region of Chiapas, Mexico (15° 11′ N, 92° 20′ W). The area receives about 4500 mm of rain annually and is located between elevations of 900 and 1150 m. According to a standard classification, the farm classifies as a commercial polyculture, with almost 100 tree species total.
17. Materials and methods are available as supporting material on *Science* Online.
18. Using 1000 coffee trees as the fixed habitat background, we drew a random number from a Poisson distribution (and truncated it to an integer) and generated the initial population density for each bush. The population density was then iterated as $N(t + 1) = RN(t)$ for one time period, the first generation (where R is the finite rate of increase). We added to the result the number of individuals as determined from another random number generated from a Poisson distribution (and truncated to an integer), and this new number iterated for the second generation.
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Supporting Online Material

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Materials and Methods
Figs. S1 to S3

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Nuclear Receptor Rev-erbα Is a Critical Lithium-Sensitive Component of the Circadian Clock

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Lithium is commonly used to treat bipolar disorder, which is associated with altered circadian rhythm. Lithium is a potent inhibitor of glycogen synthase kinase 3 (GSK3), which regulates circadian rhythm in several organisms. In experiments with cultured cells, we show here that GSK3β phosphorylates and stabilizes the orphan nuclear receptor Rev-erbα, a negative component of the circadian clock. Lithium treatment of cells leads to rapid proteasomal degradation of Rev-erbα and activation of clock gene *Bmal1*. A form of Rev-erbα that is insensitive to lithium interferes with the expression of circadian genes. Control of Rev-erbα protein stability is thus a critical component of the peripheral clock and a biological target of lithium therapy.

Genetic and biochemical analysis reveals that a 24-hour circadian rhythm is present throughout the animal kingdom (*1*–*3*). In mammals, circadian rhythm is a fundamental regulatory factor for many aspects of behavior and physiology, including sleep-wake cycles, blood pressure, body temperature, and metabolism (*1*–*3*). Disruption in circadian rhythms leads to increased incidence of many diseases, such as cancer and mental illness (*1*, *3*). Bipolar disorder in particular is associated with disturbed circadian rhythm (*4*).

Cells throughout the body also display 24-hour rhythms (*3*, *5*). These are entrained by signals from a central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus, which is reset daily by light (*3*). Cellular rhythms are generated and maintained through interconnected transcriptional feedback of clock genes (*3*, *6*). The cycle starts when two bHLH-PAS domain proteins, BMAL1 and CLOCK, heterodimerize to activate a number of clock genes including *Per1*, *Per2*, *Cry1*, and *Cry2*. As a negative feedback loop, PER and CRY accumulate in the cytosol and then translocate into the nucleus. Once inside the nucleus, the PER-CRY complex inhibits its own transcription by binding to BMAL-CLOCK (*3*, *6*–*8*). An additional negative feedback loop requires the transcription repression function of the orphan nuclear receptor Rev-erbα, which represses the transcription of *Bmal1* during circadian night and is responsible for rhythmic expression of the *Bmal1* gene (*9*–*11*). *Rev-erbα* itself is activated by BMAL1-CLOCK and thereby represents the link between the positive and negative loops of the circadian clock (*9*).

Posttranslational modifications also play an essential role in resetting the clock (*2*, *3*, *12*).

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Phosphorylation of PER by casein kinase Iε leads to its ubiquitination and proteasomal degradation and, therefore, controls the period length in mammals as well as *Drosophila* (*13*). Mutation in *Shaggy*, the *Drosophila* homolog of glycogen synthase kinase 3β (GSK3β), lengthens the circadian period (*12*, *14*), similar to the mammalian effects of lithium (*15*, *16*), a potent and selective GSK3 inhibitor (*17*). We observed that the amino terminus of Rev-erbα is serine-rich, with several potential GSK3β phosphorylation sites (fig. S1A). Remarkably, in human 293T embryonic kidney cells, reduced expression of GSK3β by small interfering RNA (siRNA) led to a near complete loss of endogenous Rev-erbα protein (Fig. 1A), as well as ectopically expressed Flag epitope–tagged Rev-erbα (fig. S2). This effect was posttranscriptional, as *Rev-erbα* mRNA was increased dramatically by loss of GSKβ (Fig. 1B), consistent with the known function of Rev-erbα protein to repress its own gene expression (*18*). *Bmal1*, the key circadian target of Rev-erbα, was also markedly induced in cells lacking GSK3β (Fig. 1B). A similar reduction in Rev-erbα protein was observed when a dominant-negative form of GSK3β was introduced into HepG2 cells by adenovirus-mediated delivery (fig. S3). These results suggest that GSK3β activity is required for stabilization of Rev-erbα protein.

Next, we asked whether modulating GSK3β activity influences Rev-erbα–mediated clock gene regulation. GSK3β is a constitutively active kinase that is inhibited by phosphorylation on serine 9 by multiple signaling pathways (*19*). We found that serum shock, which synchronizes circadian oscillations in cultured NIH3T3 mouse fibroblasts (*5*, *20*), led to the immediate and robust phosphorylation of GSK3β at serine 9 (Fig. 1C). Remarkably, the level of Rev-erbα protein plummeted during this time and recovered when the cells were returned to serum-free medium and GSK3β phosphorylation abated. The changes in the cellular Rev-erbα protein were reflected by the cyclic occupancy of the *Bmal1* gene promoter by the nuclear repressor corepressor (N-CoR) that is recruited by Rev-erbα (Fig. 1D), accompanied by transient induction of *Bmal1* and *Rev-erbα* mRNAs (Fig. 1E).

GSK3β is also inhibited by lithium (*17*). Treatment of 293T cells with 20 mM LiCl dramatically down-regulated ectopically expressed Flag epitope–tagged Rev-erbα without reducing the mRNA level (Fig. 2A; fig. S4). By contrast, endogenous β-catenin was stabilized by LiCl as expected (*21*). The destabilizing effect of lithium on Rev-erbα was prevented by treatment of the cells with MG132, an inhibitor of the 26*S* proteasome (Fig. 2A). Inhibition of proteasome activity also prevented loss of Rev-erbα due to siRNA inhibition of GSK3β (fig. S2A). Furthermore, we detected polyubiquitination of Flag–Rev-erbα (Fig. 2B), indicating that inhibition of GSK3β targets Rev-erbα for degradation by the ubiquitin-dependent proteasome pathway. Lithium treatment also reduced the association of Rev-erbα and the corepressor complex with the *Bmal1* promoter, while increasing histone acetylation (Fig. 2C) and gene expression (Fig. 2D).

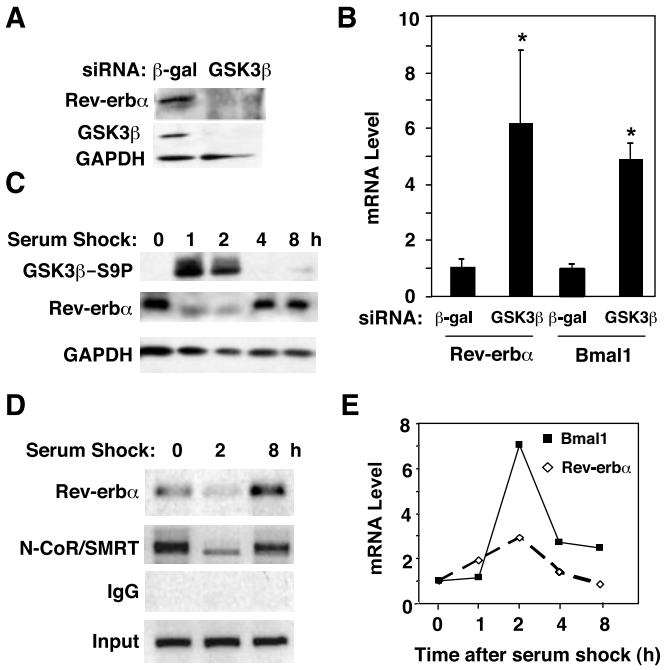


Fig. 1. GSK3β regulates Rev-erbα protein amount and function. (**A**) Immunoblots of extracts from human 293T cells transfected with siRNA vector for either β-galactosidase (β-gal) control or human GSK3β. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as loading control. (**B**) 293T cells transfected with β-gal or GSK3β siRNA vector were analyzed for *Rev-erbα* and *Bmal1* mRNA as described in methods (in the SOM). Shown is the mean ± SD of three experiments. **P* < 0.05 versus control siRNA. (**C**) Immunoblots of extracts of mouse NIH3T3 cells subjected to serum shock (exposed to medium containing 50% horse serum at time 0 then switched to medium containing 0.5% bovine serum 2 hours later). (**D**) Chromatin immunoprecipitation (ChIP) for Rev-erbα and the corepressors N-CoR/SMRT at the mouse *Bmal1* promoter in serum-shocked NIH3T3 cells. Rabbit IgG was used as nonspecific control. (**E**) Effect of serum shock on *Bmal1* and *Rev-erbα* mRNA in NIH3T3 cells.

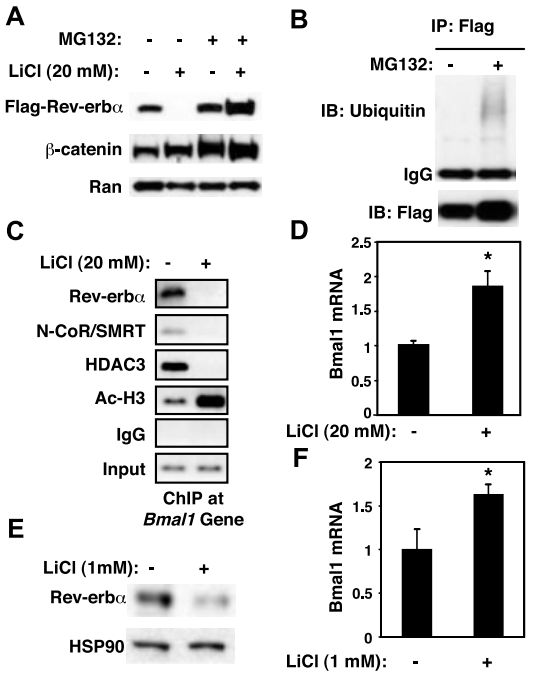


Fig. 2. Lithium reduces Rev-erbα protein amount and function. (**A**) Immunoblots of extracts from 293T cells transfected with Flag epitope–tagged Rev-erbα, and exposed to 20 mM LiCl and/or MG132. β-Catenin served as positive control for lithium effect on inhibition of GSK3β, and Ran guanosine triphosphatase (Ran GTPase) served as loading control. (**B**) Immunoprecipitation with Flag-specific antibody was performed from 293T cell extracts expressing Flag epitope–tagged Rev-erbα treated with or without MG132. The ubiquitin-conjugated Rev-erbα protein was detected by immunoblotting. (**C**) ChIP assay comparing the occupancy of endogenous Rev-erbα, N-CoR/SMRT, HDAC3, and acetylated histone (Ac-H3) at the human *Bmal1* promoter of 293T cells treated with or without LiCl (20 mM) for 16 hours. (**D**) Effect of LiCl (20 mM, 16 hours) on *Bmal1* gene expression in 293T cells. (**E**) Effect of LiCl (1 mM, 72 hours) on Rev-erbα levels in 293T cells. Heat shock protein Hsp90 served as loading control. (**F**) Effect of LiCl (1 mM, 72 hours) on *Bmal1* gene expression in 293T cells. For RNA analyses, shown are the mean ± SD of three experiments. **P* < 0.05 versus control treatment.

Although we have used lithium at a concentration of 20 mM to maximally inhibit GSK3β, chronic lithium therapy for patients with bipolar disorder aims for a serum concentration of ~1 mM (*22*). We therefore treated 293T cells with 1 mM LiCl for 72 hours and observed a marked reduction of Rev-erbα protein (Fig. 2E) and

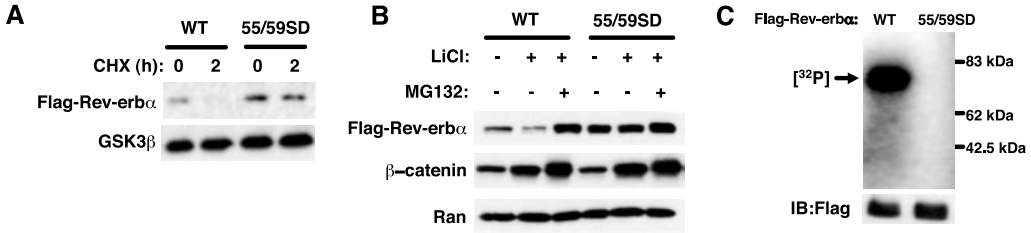
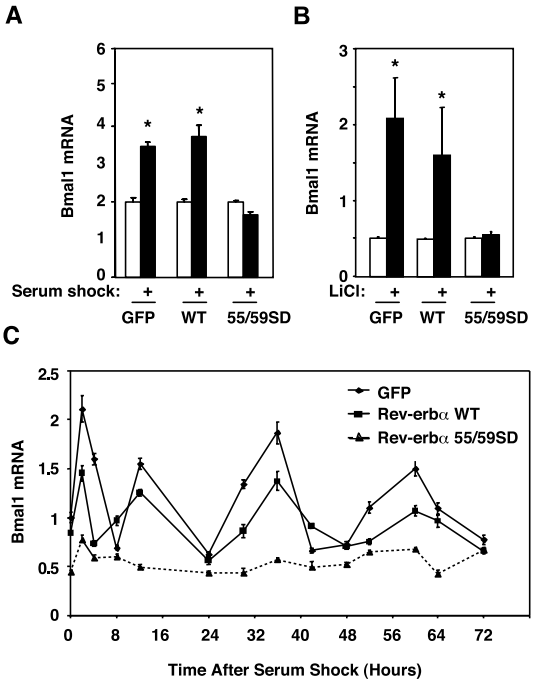


Fig. 3. Rev-erbα is stabilized by GSK3β-mediated phosphorylation of serines 55 and 59. (**A**) Immunoblots of 293T cells transfected with Flag-tagged Rev-erbα (WT or 55/59SD mutant), exposed to 25 μg/ml cycloheximide (CHX) for 2 hours. (**B**) Immunoblots of 293T cells transfected with Flag-tagged Rev-erbα and treated with or without LiCl (20 mM) and MG132 (1μM). (**C**) In vitro phosphorylation of Rev-erbα by GSK3β. HeLa cells were stably transfected with expression vector for WT or 55/59SD Flag-tagged Rev-erbα. Flag-tagged Rev-erbα protein was immunoprecipitated and incubated with [γ-^{32}P]ATP (adenosine triphosphate) and recombinant GSK3β as in the SOM. Proteins were resolved on 12% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) gel and analyzed by autoradiography (top) and immunoblot (bottom).

Fig. 4. Control of circadian gene expression by regulation of Rev-erbα protein stability. NIH3T3 cells stably expressing GFP, WT, or 55/59SD Rev-erbα were established. (**A**) Induction of mouse *Bmal1* gene expression in cells exposed to 50% horse serum for 2 hours. (**B**) *Bmal1* gene expression in cells exposed to 20 mM LiCl for 16 hours. (**C**) *Bmal1* gene expression in cells exposed to 50% horse serum for 2 hours, then switched to 0.5% horse serum for 72 hours. Shown are the mean ± SD of three experiments. *$P < 0.05$ versus control treatment.



induction of *Bmal1* gene expression (Fig. 2F). Thus, degradation of Rev-erbα occurs at a clinically relevant concentration of lithium.

Within the Rev-erbα N terminus, serine 55 and serine 59 are located in a GSK3β consensus site that is identical in human, mouse, and rat (fig. S1B). Mutation of both amino acids to negatively charged aspartate (S55D/S59D, here shortened to 55/59SD) stabilized the protein (fig. S5). The 55/59SD mutant had a longer half-life and was resistant to lithium-induced degradation (Fig. 3, A and B). An in vitro kinase assay comparing the wild type (WT) and the 55/59SD mutant as substrate for GSK3β confirmed that these two serine residues were required for phosphorylation of Rev-erbα by GSK3β (Fig. 3C).

We hypothesized that early inactivation of GSK3β, causing Rev-erbα degradation and leading to *Bmal1* induction, is a critical step for synchronization of rhythmic expression of clock genes in NIH3T3 cells. To test this, we established stable NIH3T3 cell lines expressing green fluorescent protein (GFP, control), WT Rev-erbα, or 55/59SD Rev-erbα. *Bmal1* was induced by serum shock in GFP and WT cells, but not in cells expressing the 55/59SD form of Rev-erbα (Fig. 4A). Moreover, *Bmal1* induction by lithium treatment was also absent in cells expressing the 55/59SD mutant (Fig. 4B). Lithium treatment also caused a significant change in the expression pattern of the clock gene *Per2* and the circadian output gene *Dbp* (*23*) in GFP and WT Rev-erbα–expressing cells, but not in cells expressing the Rev-erbα 55/59SD mutant (fig. S6).

We performed further analysis to determine whether Rev-erbα degradation is required for the generation and maintenance of oscillatory gene expression over several circadian cycles. Remarkably, expression of the degradation-resistant 55/59SD form, but not WT Rev-erbα, severely dampened the oscillatory expression of *Bmal1* over three circadian cycles following serum shock (Fig. 4C). Thus, GSK3β-dependent regulation of Rev-erbα is important for synchronizing and maintaining the peripheral clock.

The GSK3β homolog *Shaggy* regulates the length of the circadian period in *Drosophila* (*14*), and mammalian GSK3β enzymatic activity oscillates with a 24-hour period both in SCN and liver (*16*, *24*). Moreover, a recent report identified a single nucleotide polymorphism within the GSK3β promoter that is associated with age at onset in bipolar depression (*25*). Circadian targets of GSK3β are beginning to be elucidated, with a recent study showing that GSK3β affects nuclear entry of mPER2 (*16*). Our results demonstrate that GSK3β-dependent stabilization of Rev-erbα maintains *Bmal1* in a repressed state and, more importantly, we have shown that the inability to degrade Rev-erbα is sufficient to prevent the onset of circadian gene oscillation. One or more components in serum that mimic external endocrine and/or neural cues induce phosphorylation and inactivation of GSK3β, resulting in the degradation of Rev-erbα and initiating the cycle of gene expression (fig. S7).

Lithium influences the circadian clock in humans, and circadian rhythms are altered in patients with bipolar disorder for which lithium is a common therapy (*26*). Here, we have shown that degradation of Rev-erbα is a critical target for lithium regulation of circadian gene expression. Intriguingly, histone deacetylase activity of the N-CoR/HDAC3 (histone deacetylase 3) corepressor is inhibited by valproic acid (*27*, *28*), another mood stabilizer that modulates circadian rhythm (*29*), which suggests that this therapy may also target repression of clock genes by Rev-erbα. Given the toxicity of existing therapies, we suggest that novel approaches targeting Rev-erbα degradation may have potential in the treatment of bipolar and circadian disorders.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/311/5763/1002/DC1
Materials and Methods
Figs. S1 to S7

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On Making the Right Choice: The Deliberation-Without-Attention Effect

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Contrary to conventional wisdom, it is not always advantageous to engage in thorough conscious deliberation before choosing. On the basis of recent insights into the characteristics of conscious and unconscious thought, we tested the hypothesis that simple choices (such as between different towels or different sets of oven mitts) indeed produce better results after conscious thought, but that choices in complex matters (such as between different houses or different cars) should be left to unconscious thought. Named the "deliberation-without-attention" hypothesis, it was confirmed in four studies on consumer choice, both in the laboratory as well as among actual shoppers, that purchases of complex products were viewed more favorably when decisions had been made in the absence of attentive deliberation.

Common knowledge holds that thorough conscious thought leads to good decisions and satisfactory choices. Whether purchasing a new car, a desktop computer, or a pair of shoes, people generally believe that serious conscious deliberation increases the probability that they will make the "right" choice. This idea applies especially to choices between products that are complex, multifaceted, and expensive. Whereas most people are willing to buy a new set of towels without much thought, they are unlikely to buy a new car or outfit a new kitchen without deliberation.

A second pervasive idea is that the quality of a choice benefits from "sleeping on it." Rather than (or in addition to) thinking consciously, people usually feel that "unconscious thought" is useful for making sound decisions. Whereas conscious thought refers to thought or deliberation while conscious attention is directed at the problem at hand, unconscious thought can be defined as thought or deliberation in the absence of conscious attention directed at the problem (*1*). An example of unconscious thought is the following: One compares two holiday destinations (say the Costa Brava and Tuscany) and does not know what to decide. One puts the problem aside and after 48 hours of not thinking about it consciously, suddenly the thought "It's going to be Tuscany!" pops into consciousness. This thought itself is conscious, but the transition from indecision to a preference 2 days later is the result of unconscious thought, or of deliberation without attention.

The scientific literature has emphasized the benefits of conscious deliberation in decision making for hundreds of years (*2*, *3*). The idea that conscious deliberation is the ideal (if not always attainable) way to approach a decision forms the backbone of classic (*4*, *5*) as well as contemporary perspectives on decision making (*6*, *7*) and attitude formation (*8*, *9*). In contrast, the notion that unconscious thought is fruitful hardly developed beyond the status of "folk wisdom." It has been postulated or investigated by scientists infrequently [but see (*10*–*13*)]. The question addressed here is whether this view is justified. We hypothesize that it is not.

First, conscious thought does not always lead to sound choices. For example, participants who chose their favorite poster among a set of five after thorough contemplation showed less postchoice satisfaction than participants who only looked at them briefly (*14*, *15*). Furthermore, conscious deliberation can make multiple evaluations of the same object less consistent over time (*16*). Two reasons why conscious deliberation sometimes leads to poor judgments have been identified. First, consciousness has a low capacity (*17*, *18*), causing choosers to take into account only a subset of the relevant information when they decide (*13*, *19*). Second, conscious thought can lead to suboptimal weighting of the importance of attributes (*13*–*16*): We tend to inflate the importance of some attributes at the expense of others, leading to worse choices.

Conversely, unconscious thought, or thought without attention, can lead to good choices (*13*, *14*). In a recent experiment, participants read information about four apartments of different desirability (*20*). They were either asked to choose their favorite immediately, or given the opportunity to choose after a period of conscious thought, or distracted for some time

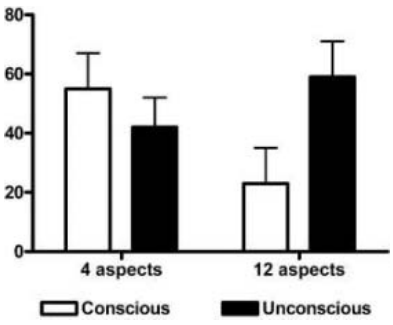

Fig. 1. Percentage of participants who chose the most desirable car as a function of complexity of decision and of mode of thought (n = 18 to 22 in each condition). Error bars represent the standard error.

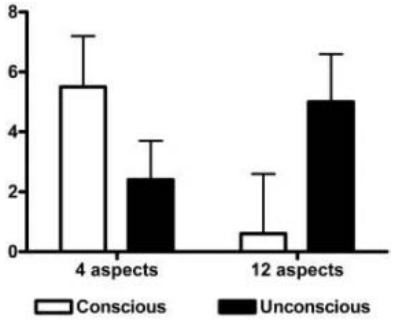

Fig. 2. Difference in attitude (on a scale of –25 to +25) toward the desirable and undesirable car as a function of complexity of decision and of mode of thought (n = 12 to 14 in each condition). Error bars represent the standard error.

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before they chose. In the third of these conditions, participants could only engage in unconscious deliberation: They knew they would have to choose later, but the distraction task prevented them from devoting conscious attention to the choice. Interestingly, unconscious thinkers made better decisions than conscious thinkers or than immediate choosers (*13*, *14*).

Recently, we formulated the Unconscious Thought Theory (UTT) (*21*) about the strengths and weaknesses of conscious thought and unconscious thought, that is, of deliberation with and without attention. Two characteristics of conscious and unconscious thought are important in the current context. First, conscious thought is rule-based and very precise (*22*, *23*). Unconscious thought can conform to rules in that it detects recurring patterns, as the literature on implicit learning shows (*24*). However, in order to actively follow strict rules, conscious attention is necessary. For example, one cannot do arithmetic without conscious attention. This capacity to follow rules makes conscious thought more precise in decision making, because it can strictly follow self-generated rules such as not exceeding a maximum price. Second, as alluded to earlier, conscious thought suffers from the low capacity of consciousness, making it less suitable for very complex issues. Unconscious thought does not suffer from low capacity. Indeed, it has been shown that during unconscious thought, large amounts of information can be integrated into an evaluative summary judgment (*13*).

These characteristics of conscious and unconscious thought led us to postulate the "deliberation-without-attention" hypothesis, on the relation between mode of thought or deliberation (conscious versus unconscious) and the complexity and quality of choice. Complexity is defined as the amount of information a choice involves. A choice between objects for which one or two attributes are important (such as oven mitts or toothpaste) is simple, whereas a choice between objects for which many attributes are important (cars or houses) is complex. Conscious thought is hypothesized, due to its precision, to lead to good choices in simple matters. However, because of its low capacity, conscious thought leads to progressively worse choices with more complex issues. Unconscious thought (i.e., deliberation without attention) is expected, because of its relative lack of precision, to lead to choices of lower quality. However, the quality of choice does not deteriorate with increased complexity, allowing unconscious thought to lead to better choices than conscious thought under complex circumstances, this latter idea being the kernel of the deliberation-without-attention hypothesis. Quality of choice was operationalized both normatively (studies 1 and 2) as well as subjectively (as postchoice satisfaction, in studies 3 and 4).

Study 1. Participants were subjected to a 2 (mode of thought: conscious versus unconscious) × 2 (complexity of choice problem: simple versus complex) factorial design (*25*). All participants read information about four hypothetical cars. Depending on the condition, each car was characterized by 4 attributes (simple) or by 12 attributes (complex). The attributes were either positive or negative. One car was characterized by 75% positive attributes, two by 50% positive attributes, and one by 25% positive attributes (supporting online text). After reading the information about the four cars, participants were assigned either to a conscious thought condition or to an unconscious thought condition. In the conscious thought condition, participants were asked to think about the cars for 4 min before they chose their favorite car. In the unconscious thought condition, participants were distracted for 4 min (they solved anagrams) and were told that after the period of distraction they would be asked to choose the best car.

The percentages of participants who chose the best car are shown in Fig. 1. The crucial two-way interaction supporting the deliberation-without-attention hypothesis was significant [$F(1,76) = 4.85$, $P < 0.04$]. Unconscious thinkers fared relatively well and showed no differences between conditions ($F < 1$, not significant). Conscious thinkers generally made the proper choice under simple conditions, but performed poorly under complex circumstances [$F(1,40) = 4.95$, $P < 0.04$].

Study 2. For the second study we made one change (*25*). Rather than asking for a choice, we asked participants about their attitudes toward each of the four cars. As the dependent variable, we used the difference in attitude toward the best car and the worst car. Again, conscious thinkers were better able to differentiate the quality of the cars under simple conditions, whereas unconscious thinkers were better able to differentiate the quality of the cars under complex conditions [$F(1,47) = 5.63$, $P < 0.03$]. The means are shown in Fig. 2.

Study 3. In a pilot study, undergraduate students were asked how many aspects of a product they would take into account in the purchase of 40 different products. In this way, we obtained an average "complexity score" for 40 different products (supporting online text).

For the actual study, other students were presented with this list of 40 products. From the list, they were asked to choose a product that they had recently purchased and were asked the following questions: Which product did you purchase? Did you know the product before you went on the shopping trip? How much did you think about the product between seeing it for the first time and buying it? How satisfied are you with the product?

To test our hypothesis, we distinguished participants who thought (either consciously or unconsciously) about their purchase from impulse buyers who did not think much at all. Hence, participants who indicated that they bought a product they had never come across before the shopping trip were not included, leaving only participants who knew the product beforehand ($n = 49$).

It is impossible to know whether people are engaged in unconscious thought by asking them, so strictly speaking, we can only test the relationship between conscious thought,

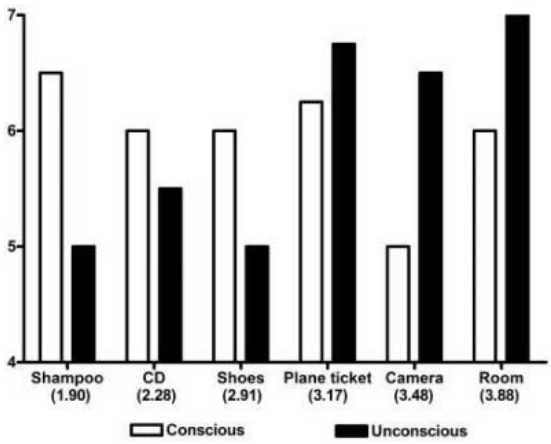

Fig. 3. The relation between mode of thought and postchoice satisfaction (on a scale of 1 to 7) for the six products most frequently chosen in study 3. Higher bars indicate more satisfaction. The more complex the product (on a scale of 1 to 5), the further to the right it is shown. The complexity score is given in parentheses. Participants were divided into conscious and unconscious thinkers on the basis of a median-split for each product individually. Each bar represents between two and five participants.

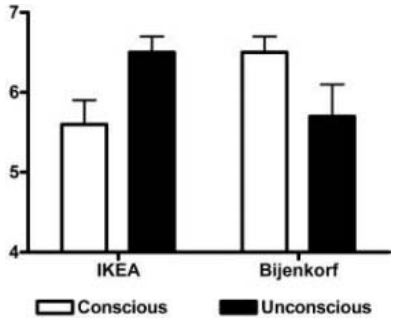

Fig. 4. Postchoice satisfaction of IKEA ($n = 27$) and Bijenkorf ($n = 27$) shoppers as a function of mode of thought. Error bars represent the standard error.

complexity, and quality. However, it follows from our definition of conscious and unconscious thought (according to which attention to the problem at hand is the crucial distinguishing factor) that they are at least partly dependent. At any one point in time, attention is either directed at the decision under consideration, or it is not; that is, at any particular point in time, either you are attending to buying a car, or you are not. The more you think about a decision consciously (that is, with attention), the less time remains to think about the same decision unconsciously (that is, without attention).

We regressed the amount of thought and the average number of aspects on postchoice satisfaction. As expected, thinking does not make people more satisfied, nor does complexity (t's < 1). However, the interaction of the two parameters significantly predicted postchoice satisfaction [$t(48) = 2.13$, $P < 0.04$]. Correlations were calculated between amount of thought and postchoice satisfaction for three categories of products: complex, medium, and simple. For products of medium complexity, no correlation was found [$r(18) = -0.03$]; for simple products, a positive correlation was found [$r(15) = 0.57$, $P < 0.03$]; and for complex products, a negative correlation was found [$r(16) = -0.56$, $P < 0.03$]. As expected, the more people thought consciously about simple products, the more satisfied they were with their purchase. Conversely, the more people thought consciously about complex products, the less satisfied they were with their purchase. Figure 3 depicts satisfaction as a function of mode of thought for the six most frequently chosen products (*26*).

Study 4. On the basis of the pilot study to study 3, two shops were selected: one where people generally buy complex products (IKEA, which sells mainly furniture) and one where people generally buy simple products (Bijenkorf, a department store like Macy's that sells clothes, clothing accessories, and kitchen accessories). At the exit, shoppers were asked the following questions: What did you buy? How expensive was it? Did you know the product before you went on the shopping trip? and How much did you think about the product between seeing it for the first time and buying it? A few weeks later, the shoppers were asked (over the phone) how satisfied they were with their purchases. As in study 3, participants who indicated that they bought a product they had never come across before the shopping trip were not included.

We divided participants ("thinkers") on the basis of a median-split procedure into those who engaged in much conscious thought (conscious thinkers) and those who engaged in little conscious thought (unconscious thinkers). As expected, conscious thinkers reported more postchoice satisfaction than unconscious thinkers for Bijenkorf products (simple products) [$F(1,25) = 6.52$, $P < 0.02$]. The opposite was true for the IKEA customers (complex products), in which case unconscious thinkers showed more postchoice satisfaction than conscious thinkers [$F(1,25) = 6.12$, $P < 0.02$] (Fig. 4).

In sum, in four studies we demonstrated the deliberation-without-attention effect. Conscious thinkers were better able to make the best choice among simple products, whereas unconscious thinkers were better able to make the best choice among complex products. Among people who knew the product they purchased before they went on a shopping trip, the amount of conscious thought was positively related to postchoice satisfaction for simple products and negatively related to postchoice satisfaction for complex products.

Our aim was to test the "deliberation-without-attention" hypothesis both in the laboratory and among shoppers. In that sense, it is important to view our set of studies as a whole rather than as a series of individual studies. Study 4 has unavoidable disadvantages such as that the IKEA and Bijenkorf samples may have differed (after all, different shops attract a different clientele), which naturally opens the potential for alternative explanations. Therefore, study 3 was done in order to "bridge" the laboratory studies with study 4. It has many of the assets of study 4 (real choices between real products with satisfaction as the dependent variable), except that all participants were students.

Although we investigated choices among consumer products in our studies, there is no a priori reason to assume that the deliberation-without-attention effect does not generalize to other types of choices—political, managerial, or otherwise. In such cases, it should benefit the individual to think consciously about simple matters and to delegate thinking about more complex matters to the unconscious.

References and Notes

1. It is important to note that attention to the problem at hand is the crucial distinction in our definitions of conscious and unconscious thought. Thinking about buying a new car while attention is directed at possible new cars is conscious thought. Thinking about buying a new car while attention is temporarily directed elsewhere is unconscious thought. This distinction does not mean that conscious thought only comprises conscious processes. One can compare it to speech. Speech is a conscious process (i.e., attention is directed at it while one speaks), but it is in part dependent on accompanying unconscious processes (such as processes responsible for syntax or word choice).
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10. As far as we know, the possibility of unconscious thought (as well as the term "unconscious thought") was explicitly used for the first time by Schopenhauer, who remarked that "One might almost believe that half of our thinking takes place unconsciously" (*27*).
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20. Quality of decision was operationalized from a normative perspective. One of the choice options was made more desirable than the others because it had been assigned more positive aspects than the other apartments.
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24. Unconscious thought is reminiscent of implicit learning, but there is an important difference. Implicit learning refers to aspects of a task that are learned while working on the task (and that are inaccessible to consciousness). Unconscious thought refers to thought processes that take place after the encoding of relevant information. A good example of this definition of unconscious thought is the groundbreaking work by Stickgold and colleagues on learning during sleep. See, e.g., (*30*, *31*).
25. Materials and methods are available as supporting material on *Science* Online.
26. We found a correlation between number of aspects and amount of thought ($r = 0.54$, $P < 0.001$): The more complex a product is, the more people think consciously when deciding to purchase it. Understandable as this may be, our analysis suggests that people should do the opposite, i.e., think unconsciously when deciding to purchase a complex product. The correlation between number of aspects and price was also significant ($r = 0.45$, $P < 0.001$): Expensive products were more complex than inexpensive ones.
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Supporting Online Material

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Activity-Dependent Regulation of MEF2 Transcription Factors Suppresses Excitatory Synapse Number

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In the mammalian nervous system, neuronal activity regulates the strength and number of synapses formed. The genetic program that coordinates this process is poorly understood. We show that myocyte enhancer factor 2 (MEF2) transcription factors suppressed excitatory synapse number in a neuronal activity- and calcineurin-dependent manner as hippocampal neurons formed synapses. In response to increased neuronal activity, calcium influx into neurons induced the activation of the calcium/calmodulin-regulated phosphatase calcineurin, which dephosphorylated and activated MEF2. When activated, MEF2 promoted the transcription of a set of genes, including *arc* and *synGAP*, that restrict synapse number. These findings define an activity-dependent transcriptional program that may control synapse number during development.

During nervous system development, synaptic activity influences the strength and number of synapses that form between neurons. Although much has been learned about the molecular mechanisms that influence local changes in synaptic strength, less is known about how neuronal activity regulates the number of synaptic connections a neuron will form and maintain.

Neuronal activity modulates synapse number both during brain development and in adult organisms. The number of synapses formed during development can be in excess of the number that is maintained into adulthood (*1*, *2*). These excess synapses are eliminated in a manner that depends on sensory experience and synaptic activity. Similarly, the induction of long-term potentiation (LTP) and long-term depression (LTD) in the mature hippocampus lead to the formation and deconstruction, respectively, of spine synapses (*3*, *4*). Because the activity-dependent remodeling of synapses is often coordinated throughout an entire neuron (*5*), a global activity-dependent process may operate to control synapse number.

Neurons can coordinate activity-dependent processes throughout the cell through the activation of new gene transcription (*6*). The influx of calcium into neurons in response to synaptic activity leads to the activation of a number of transcription factors. Members of the myocyte enhancer factor 2 family (MEF2A to MEF2D) of transcription factors are highly expressed in the brain and are tightly regulated by several distinct calcium signaling pathways (*7*, *8*). Here, we report that MEF2 transcription factors act as negative regulators of synapse number in hippocampal neurons.

In rat brain lysates, MEF2A and MEF2D proteins were detected during embryonic stages, but their expression increased during the first 3 weeks after birth, a time during which synapses form and are remodeled (*9*) (fig. S1). Because the developmental regulation of MEF2 expression was recapitulated in dissociated hippocampal cultures (fig. S1), we used RNA interference (RNAi) to investigate the function of MEF2 proteins in culture. Short hairpin RNAs (shRNAs) that target MEF2A and MEF2D caused the persistent depletion of these proteins in the nuclei of transfected hippocampal neurons over a period of at least 10 days (Fig. 1A and fig. S2). The membrane depolarization-induced expression of a luciferase reporter gene under the control of three consensus MEF2 response elements (MREs) was also reduced by the reduction of MEF2A and MEF2D (Fig. 1B). However, the membrane depolarization-induced expression of a cyclic adenosine monophosphate response element (CRE) reporter gene was unaffected by MEF2 RNAi (fig. S3).

To investigate whether MEF2 regulates the number of synaptic contacts a neuron receives, we transfected shRNAs into cells at a time when synapses are just beginning to form [day in vitro (DIV) 5] and analyzed the number of synapses that had formed 8 to 10 days later. We used a low-efficiency method of transfection so that we could examine synapses formed onto transfected neurons by untransfected neurons in the same culture. Cells were fixed and stained for the presynaptic marker synapsin-1, as well as the postsynaptic marker postsynaptic density protein 95 (PSD-95). These proteins were localized to discrete puncta that frequently overlapped, reflecting the clustering of these proteins at synaptic sites (Fig. 2A). We counted the number of sites where these puncta were colocalized

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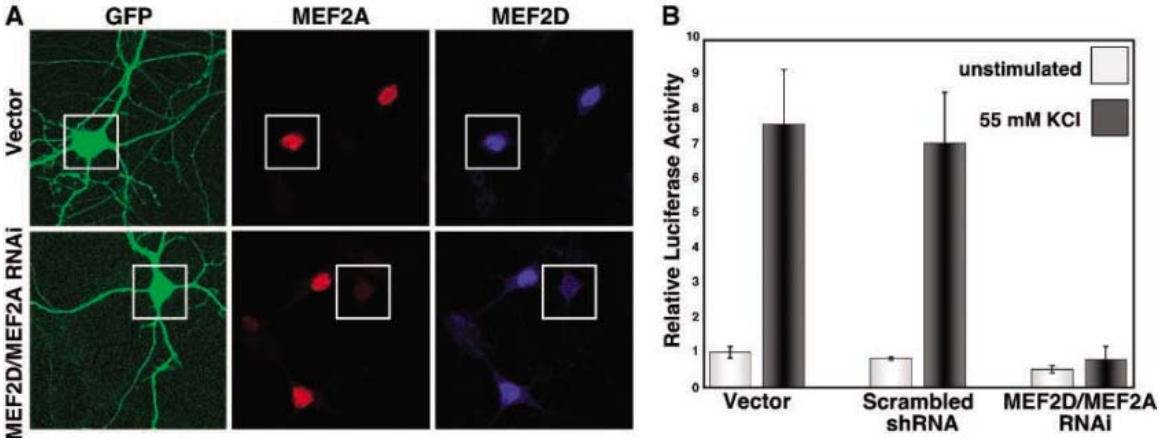

Fig. 1. Reduction of MEF2A and MEF2D attenuates MEF2-dependent transcription in hippocampal neurons. (**A**) Images of hippocampal neurons transfected with the indicated shRNA expression plasmids and stained with antibodies to MEF2A (red) and MEF2D (blue). Cells were transfected at embryonic day 18 (E18) + 5 DIV and fixed at E18 + 14 DIV. Transfected cells indicated by boxes. (**B**) Luciferase reporter assay with the use of 3xMRE-Luc cotransfected with the indicated shRNA expression plasmids. Two days after transfection, cells were pretreated with AP5 and tetrodotoxin (TTX) for 1 hour before depolarization with 55 mM KCl for 7 hours. Data are from two independent experiments, each of which was conducted in triplicate, and are means ± standard error of the mean (SEM) for unstimulated cells and +SEM for cells treated with KCl.

(henceforth referred to as cocluster density or synapse number) along the dendrites of transfected cells. Reduction of MEF2A and MEF2D resulted in a significant increase in the number of synapses formed onto neurons (Fig. 2B), suggesting that MEF2 proteins function to restrict the number of synapses received by a neuron. The effect of MEF2 reduction on synapse number is due to the loss of MEF2-dependent transcription, given that the expression of a constitutively active and RNAi-resistant form of MEF2 (MEF2-VP16) completely reversed the increase in synapse number caused by MEF2 RNAi (Fig. 2B). A DNA-binding deficient mutant (MEF2ΔDBD-VP16) did not alter synapse number, indicating that this effect is dependent on the ability of MEF2-VP16 to bind to DNA and activate transcription.

Because MEF2 proteins promote calcium-dependent neuronal survival in cerebellar granule neurons deprived of growth factors (*8*), we cultured our hippocampal neurons under conditions in which MEF2 is not required for survival. We also monitored cell viability by assessing the dendritic morphology and passive membrane properties of neurons that were transfected with control or MEF2-specific shRNAs and found no detectable differences (figs. S4 and S5).

To test whether the synapses formed on MEF2 shRNA-expressing cells were functional, we recorded miniature excitatory postsynaptic currents (mEPSCs) from neurons 8 to 10 days after transfection in a whole-cell voltage clamp configuration. The reduction of MEF2A and MEF2D caused a significant increase in mEPSC frequency, whereas the expression of a scrambled shRNA had no effect (Fig. 2C). We observed no effect of MEF2A and MEF2D RNAi on mEPSC amplitude or the mEPSC waveform, indicating that, apart from the effect on mEPSC frequency, the AMPA receptor–mediated postsynaptic responses to spontaneous glutamate release were unaffected in these cells (fig. S6). The MEF2 RNAi-dependent increase in mEPSC frequency was reversed by cotransfection with MEF2-VP16 but not MEF2ΔDBD-VP16, suggesting that increased MEF2-dependent transcription can reverse the effect of MEF2 RNAi on mEPSC frequency (Fig. 2C).

Presynaptic alterations might account in part for the increase in mEPSC frequency in MEF2 shRNA-expressing cells, but we also observed an increase in the number of excitatory synapses by immunocytochemistry, which suggests that an increase in synapse number likely contributes to this effect. The increase in mEPSC frequency could also be due in part to an increase in the size or strength of preexisting synapses so that mEPSCs below our threshold of detection become detectable in cells treated with MEF2 RNAi. However, no difference was detected in the distributions of mEPSC amplitudes between control and MEF2 shRNA-expressing cells, suggesting that this possibility is unlikely.

To test whether activation of MEF2 is sufficient to suppress excitatory synapse number after synapses have already formed, we used an inducible gene-activation system in which the ligand-binding domain of the estrogen receptor (ER) is fused to the C terminus of MEF2-VP16. The ER fragment is mutated so that it responds specifically to 4-hydroxytamoxifen (4OHT). In the absence of 4OHT, MEF2-VP16–ER remained sequestered in the cytoplasm of transfected hippocampal neurons, but it rapidly translocated to the nucleus and activated MEF2-dependent transcription upon addition of 4OHT to the culture media (fig. S7 and Fig. 3A).

We transfected MEF2-VP16–ER into hippocampal neurons and allowed synapses to develop in the absence of 4OHT. After synapses had formed (DIV 20 and 21), cells were fixed either before or after 12 or 24 hours of 4OHT treatment. We determined the density of synapsin-1–PSD-95 coclusters and also monitored the number of dendritic spines per unit length of dendrite. Dendritic spines are the primary sites of excitatory contacts in mature hippocampal neurons and provide an additional measure of the number of excitatory synapses (*10*). In neurons transfected with an empty vector or a MEF2ΔDBD-VP16–ER expression vector, the density of synapsin-1–PSD-95 coclusters and dendritic spines remained unchanged over a 12- or 24-hour time period when cells were treated with 4OHT (Fig. 3B and fig. S8). However, neurons expressing MEF2-VP16–ER displayed a significant decrease in both cocluster density and spine number after 12 or 24 hours of 4OHT treatment. These results indicate that the activation of MEF2-dependent transcription is sufficient to restrict synapse number.

MEF2 is activated by diverse extracellular stimuli, including growth factors, neurotrophins such as brain-derived neurotrophic factor (BDNF), and calcium influx resulting from neuronal activity (*7*). Because glutamate release at excitatory synapses is thought to play a critical role in synaptic remodeling, we tested whether the ability of MEF2 to suppress synapse number requires glutamate-stimulated calcium influx. We transfected a MRE reporter plasmid into hippocampal neurons at DIV 10. To block calcium influx resulting from spontaneous glutamatergic synaptic transmission, we exposed hippocampal cultures to the *N*-methyl-D-aspartate–type glutamate receptor (NMDAR) antagonist 2-amino-5-phosphonopentanoic acid (AP5) or the L-type voltage-gated calcium channel (VGCC) antagonist nimodipine and found that either treatment led to a decrease in expression of the MRE reporter gene (Fig. 4A). Conversely, application of glutamate caused an

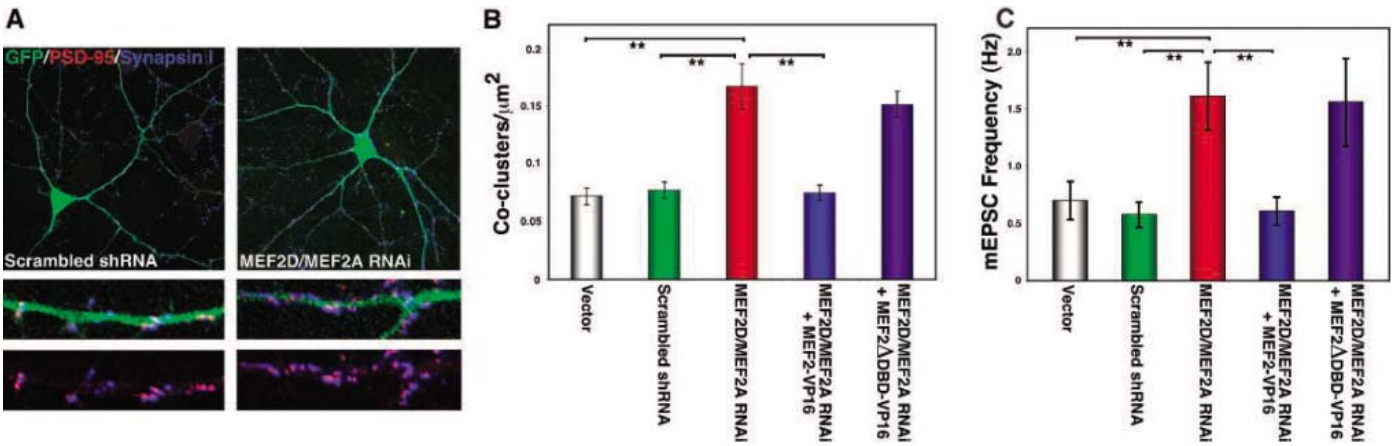

Fig. 2. Increased synapse number and mEPSC frequency after reduction of MEF2 in hippocampal neurons. (**A**) (Top) Images of hippocampal neurons transfected with the indicated plasmids and stained with antibodies to PSD-95 (red) and synapsin-1 (blue). (Bottom) Details of dendrites with and without cotransfected green fluorescent protein (GFP) displayed. (**B**) Average density of synapsin-1–PSD-95 coclusters along the dendrites of neurons transfected with the indicated plasmids. $P < 0.001$, analysis of variance (ANOVA); asterisks indicate statistical significance in pairwise comparison: $P < 0.001$, Bonferroni-Dunn post-hoc test. $n \geq 14$ cells per condition (137 total, including data shown in fig. S18) and data are means ± SEM. (**C**) Average mEPSC frequency for cells transfected with the indicated plasmids. $P < 0.01$, ANOVA; asterisks indicates statistical significance in pairwise comparison: $P < 0.005$, Bonferroni-Dunn post-hoc test. $n \geq 8$ cells per condition (55 total) and data are means ± SEM.

NMDAR-dependent increase in reporter gene expression, whereas membrane depolarization caused by increased extracellular concentration of potassium chloride (KCl) induced expression of the MRE reporter gene in a manner that required L-type VGCC-mediated calcium influx (Fig. 4, B and C). These data indicate that MEF2 transcriptional activity is induced by calcium influx through NMDARs and L-type VGCCs in hippocampal neurons.

To determine whether neuronal activity-dependent induction of MEF2 is required for the ability of MEF2 to suppress synapse number, we blocked synaptic activity by exposing cultured hippocampal neurons to the glutamate receptor antagonists AP5 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and tested whether MEF2 functioned to restrict synapse number under these conditions. Blockade of synaptic activity does not alter the density of synapses formed onto control transfected hippocampal neurons (*11*). This likely reflects the evidence that activity is required for both the strengthening and weakening of synapses in these cultures. However, the reduction of MEF2A and MEF2D failed to cause an increase in the density of synapsin-1–PSD-95 coclusters in the presence of AP5 and CNQX (Fig. 4D). Thus, the ability of MEF2 to suppress synapse number requires the presence of glutamatergic synaptic activity, suggesting that the regulation of MEF2 by synaptic activity may be critical to its ability to suppress synapse number.

Because MEF2 phosphorylation is known to regulate its transcriptional activity (*7*), we tested whether phosphorylation of MEF2 is critical for calcium-dependent MEF2 activation in hippocampal neurons. In the absence of glutamatergic synaptic activity, we detected multiple species of MEF2A and MEF2D proteins in extracts from hippocampal neurons (Fig. 4E). The slower migrating forms of MEF2 appeared to be due to phosphorylation, because treatment of lysates from nondepolarized hippocampal cells with alkaline phosphatase led to the disappearance of these species. Membrane depolarization of hippocampal neurons also led to the disappearance of the slowly migrating forms of MEF2 in a manner that required calcium influx, suggesting that neuronal activity-induced calcium influx leads to the dephosphorylation of MEF2A and MEF2D. The calcium- and calmodulin-regulated phosphatase calcineurin (PP2B) has been implicated in the activity-dependent dephosphorylation of MEF2 (*12*). Using the specific inhibitors of calcineurin, cyclosporin A (CsA) and FK506, we observed that the membrane depolarization-induced dephosphorylation of MEF2A and MEF2D and MRE reporter gene expression were largely blocked by inhibiting calcineurin activity (Fig. 4, C and E). Moreover, MEF2D immunoprecipitated from nondepolarized neurons was dephosphorylated by recombinant calcineurin in vitro (fig. S9). These data suggest that calcineurin dephosphorylates MEF2 at specific sites that, when phosphorylated, inhibit the ability of MEF2 to activate transcription.

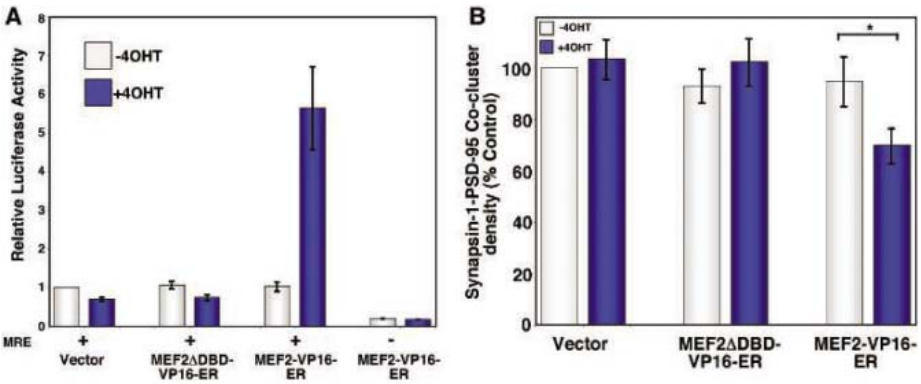

Fig. 3. Synapse loss after activation of MEF2-dependent transcription. (**A**) Luciferase reporter assay using 3xMRE-Luc or 3xMREmut-Luc cotransfected with the indicated expression plasmids. Two days after transfection, cells were stimulated with 4OHT for 6 hours. Data are from two independent experiments, each of which was conducted in triplicate, and are means ± SEM. (**B**) Average synapsin-1–PSD-95 cocluster density along the dendrites of neurons transfected and stimulated in the indicated manner for 24 hours. $P < 0.01$, ANOVA; asterisk indicates statistical significance in pairwise comparison: $P < 0.01$, Fisher's protected least significance difference (PLSD) test. $n \geq 21$ cells per condition (138 total); data are from three independent experiments and are means ± SEM.

To identify the sites of dephosphorylation, we used mass spectrometry and an analysis of MEF2 sequence conservation across species, which led us to focus on three serine residues in MEF2A and MEF2D—Ser^{221}, Ser^{255}, and Ser^{408} (on the basis of their positions in human MEF2A). Phosphorylation at Ser^{408} inhibits MEF2 activity in neurons (*13*). We generated three separate phosphospecific antibodies that recognize MEF2A when phosphorylated at Ser^{221}, Ser^{255}, or Ser^{408} (fig. S10). Membrane depolarization of hippocampal neurons resulted in a decrease in the phosphorylation of each of these three sites in a manner that was dependent on calcineurin activation (fig. S11). Application of BDNF, which activates MEF2 in neurons (*14*), did not detectably alter the phosphorylation of MEF2 at Ser^{221}, Ser^{255}, and Ser^{408} (fig. S12), suggesting that the dephosphorylation of these sites by calcineurin might confer a calcium-specific activation signal to promoters occupied by MEF2.

To test whether dephosphorylation of MEF2A at Ser^{221}, Ser^{255}, and Ser^{408} is important for neuronal activity-induced MEF2 transcription in neurons, we generated mutants of MEF2A in which an alanine replaced serine at all three serine residues, or Ser^{408} alone (fig. S13). We developed a protein replacement assay in which the expression of endogenous MEF2A and MEF2D in neurons was almost completely eliminated by RNAi, and MEF2 transcriptional activity was restored by expressing a MEF2A or MEF2D cDNA bearing silent mutations in the region targeted by the co-expressed shRNA, which renders the MEF2s resistant to RNAi (RiR) (Fig. 4F and fig. S14). MEF2 cDNAs lacking these silent mutations were still susceptible to RNAi and did not restore MEF2 activity. In this assay, both the triple mutant (Ser^{221}→Ala, Ser^{255}→Ala, and Ser^{408}→Ala) and the Ser^{408}→Ala mutant restored reporter gene expression to a greater extent than did wild-type MEF2A-RiR, indicating that phosphorylation of MEF2 at these serine residues inhibits MEF2 activity in neurons (Fig. 4F). In contrast, mutations of two highly conserved threonine residues that participate in stimulus-dependent MEF2 activation in other contexts did not alter the ability of MEF2A-RiR to restore calcium-dependent MEF2 activation (figs. S12 and S13) (*15*). Taken together, these data indicate that the calcium-dependent activation of MEF2 in neurons is distinct from these previously characterized mechanisms and requires the calcineurin-dependent dephosphorylation of MEF2.

We next tested whether, under culture conditions where calcineurin activity was inhibited so that MEF2 would not be dephosphorylated and activated, the reduction of MEF2A and MEF2D still caused an increase in synapse number. Chronic treatment of hippocampal neurons with CsA and FK506 led to a modest increase in synapse number (Fig. 4G). However, the reduction of MEF2A and MEF2D did not cause a significant increase in synapsin-1–PSD-95 cocluster number in the presence of calcineurin inhibitors (Fig. 4G). This suggests that the ability of MEF2 to suppress synapse number requires the neuronal activity-dependent activation of calcineurin and likely the dephosphorylation of MEF2.

To test whether MEF2 promotes the transcription of genes whose function is to negatively regulate the number of excitatory synapses that form onto neurons, we sought to identify activity-regulated genes whose expression is controlled by MEF2. We used lentiviral delivery

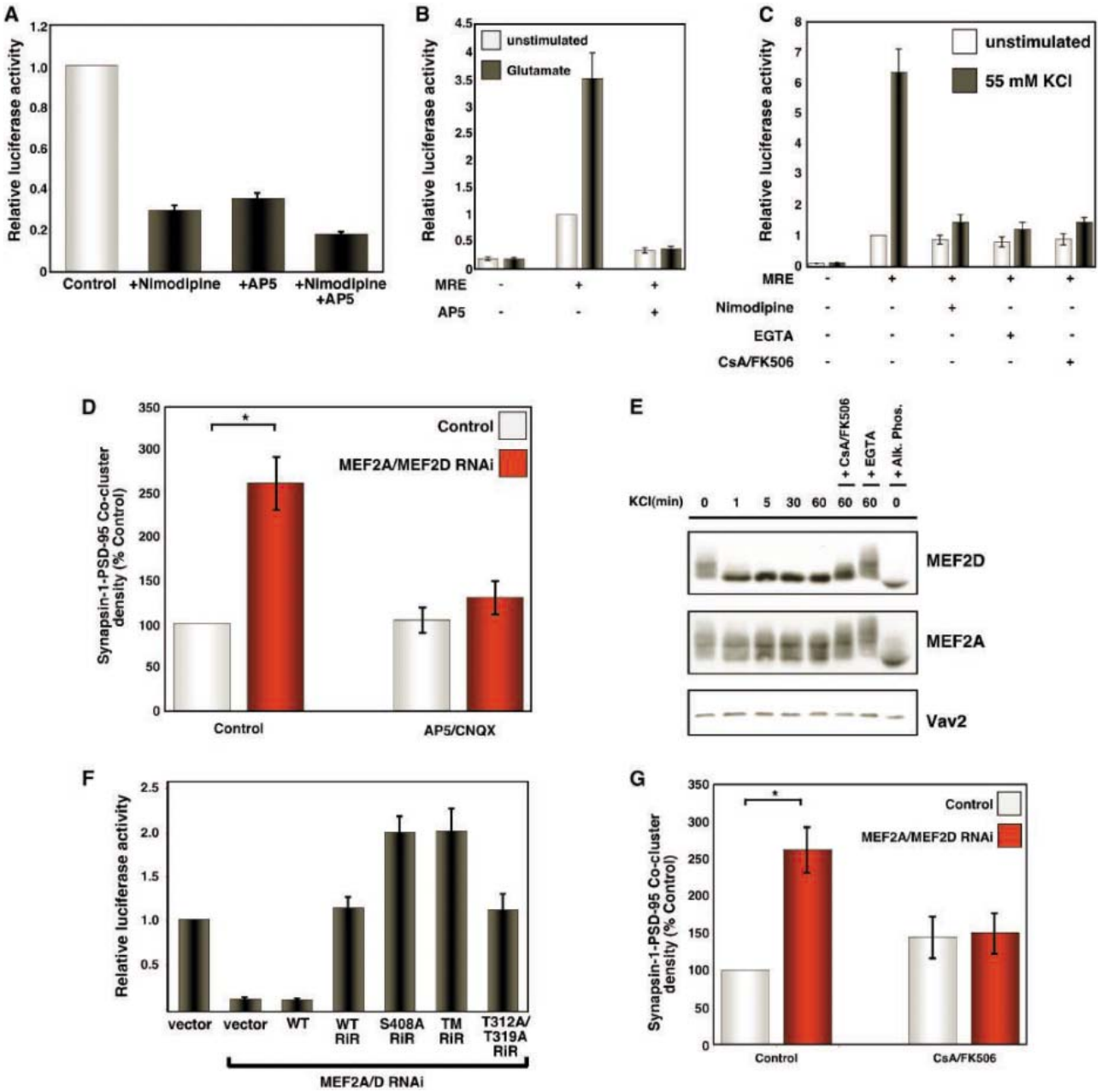

Fig. 4. Activity-dependent regulation of MEF2 by calcineurin. (**A**) Luciferase reporter assay with the use of 3xMRE-Luc transfected into E18 + 10 DIV hippocampal neurons. The indicated antagonists were added for 6 hours before lysates were collected. Data are means + SEM. (**B**) Luciferase reporter assay with the use of 3xMRE-Luc (MRE+) or 3xMREmut-Luc (MRE–). Transfected neurons were stimulated with glutamate (20 μM) for 4 hours at E18 + 10 DIV. Data are means ± SEM for unstimulated cells and means + SEM for glutamate. (**C**) Luciferase reporter assay using the same plasmids as in (B). Hippocampal neurons were transfected at E18 + 6 DIV and stimulated as in Fig. 1B. Data are means ± SEM for unstimulated cells and means + SEM for cells treated with KCl. (**D**) Density of synapsin-1–PSD-95 coclusters along the dendrites of neurons transfected with either a scrambled shRNA (control) or MEF2-specific shRNAs. $n \geq 12$ cells per condition (59 total) and data are means ± SEM. (**E**) Western blot analyses of MEF2 proteins extracted from hippocampal neurons at E18 + 8 DIV. Cells were pretreated with AP5 and TTX and stimulated with 55 mM KCl for the indicated times. (**F**) Luciferase reporter assay with the use of the same reporter plasmids as in (A), cotransfected with the indicated plasmids into hippocampal neurons at E18 + 4 to 5 DIV. Data are means + SEM. (**G**) Density of synapsin-1–PSD-95 coclusters onto neurons transfected with either scrambled shRNA (control) or MEF2-specific shRNAs. $n \geq 15$ cells per condition (63 total) and data are means ± SEM. For (D) and (G), $P < 0.001$, ANOVA; asterisks indicate statistical significance in pairwise comparison: $P < 0.001$, Bonferroni-Dunn post-hoc test. For (A) to (C) and (F), data are from two independent experiments, each of which was conducted in triplicate.

of shRNAs and Affymetrix microarrays to conduct a genome-wide screen for genes transcribed in response to membrane depolarization that are reduced in expression after reduction of MEF2. The reduction of MEF2A and MEF2D caused a decrease in the expression of genes belonging to several functional categories, including additional transcriptional regulators and signaling molecules such as kinases. We investigated two of these genes, the *activity-regulated cytoskeletal-associated protein* (*arc*) and *synaptic Ras guanosine triphosphatase activating protein* (*synGAP*), because the protein products of these genes negatively regulate synaptic development. Arc promotes the internalization of gluta-

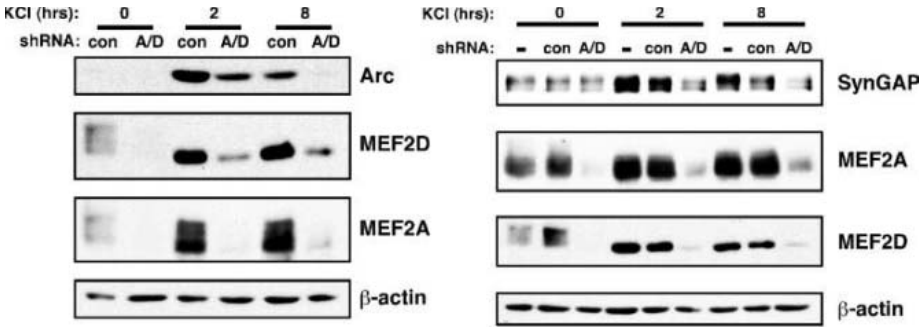

Fig. 5. Regulation of arc and synGAP by MEF2. Western blot analyses of arc (left) and synGAP expression (right) with the use of antibodies specific to the two proteins. Protein lysates were collected from neurons at E18 + 10 DIV. Neurons were transduced with lentiviruses at E18 + 3 DIV and depolarized with high KCl as in Fig. 1B before lysis. For synGAP expression analysis, neurons were lysed at E18 + 14 DIV. con, control; A/D, MEF2A and MEF2D RNAi.

mate receptors (*16*), whereas synGAP inhibits Ras–mitogen-activated protein kinase signaling in the postsynaptic compartment (*17*). We confirmed that MEF2 reduction leads to a decrease in the expression of *arc* and *synGAP* by real-time quantitative polymerase chain reaction and Western blotting (fig. S15 and Fig. 5). Moreover, we identified evolutionarily conserved regions at the genomic loci of both *arc* and *synGAP* and found that several of these DNA fragments were enriched in MEF2 chromatin immunoprecipitates (fig. S16). Thus, *arc* and *synGAP* may be direct transcription targets of MEF2.

Our results show that MEF2 transcription factors negatively regulate excitatory synapse number in an activity-dependent manner during synaptic development in vitro. They support a model in which the calcium- and calmodulin-regulated phosphatase calcineurin dephosphorylates and activates MEF2, which in turn promotes the remodeling of synapses by inducing a program of gene transcription that may involve *arc* and *synGAP*. Given that calcineurin is also required for the expression of certain forms of LTD (*18*), it is possible that MEF2 might also contribute to LTD. Whether MEF2 is important in vivo for such processes as synapse elimination, homeostatic control of synapse number, and/or LTD remains to be determined.

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Supporting Online Material

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A Calcium-Regulated MEF2 Sumoylation Switch Controls Postsynaptic Differentiation

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Postsynaptic differentiation of dendrites is an essential step in synapse formation. We report here a requirement for the transcription factor myocyte enhancer factor 2A (MEF2A) in the morphogenesis of postsynaptic granule neuron dendritic claws in the cerebellar cortex. A transcriptional repressor form of MEF2A that is sumoylated at lysine-403 promoted dendritic claw differentiation. Activity-dependent calcium signaling induced a calcineurin-mediated dephosphorylation of MEF2A at serine-408 and, thereby, promoted a switch from sumoylation to acetylation at lysine-403, which led to inhibition of dendritic claw differentiation. Our findings define a mechanism underlying postsynaptic differentiation that may modulate activity-dependent synapse development and plasticity in the brain.

The MEF2 family of transcription factors is highly expressed in the brain when neurons undergo dendritic maturation and synapse formation (*1*). MEF2A is especially abundant in granule neurons of the cerebellar cortex throughout the period of synaptogenesis (*1*) (fig. S1). In view of reported functions for transcription factors in distinct aspects of dendritic morphogenesis (*2–4*), we investigated a potential role of MEF2A in synaptic dendritic development in the cerebellar cortex.

During cerebellar development, granule neuron dendritic morphogenesis culminates in the differentiation of dendritic claws on which mossy fiber terminals and Golgi neuron axons form synapses (*5–8*). To visualize granule neurons undergoing postsynaptic differentiation, we transfected organotypic cerebellar slices prepared from postnatal day 9 (P9) rat pups with an expression plasmid encoding green fluorescent protein (GFP) (*9*). Transfected granule neurons in the internal granule layer had the typical small cell body with associated parallel axonal fibers and few dendrites (Fig. 1, A and B). Many dendrites harbored structures with the appearance of dendritic claws that were identified on the basis of classic descriptions as (i) located at the end of a dendrite, (ii) having cuplike or sicklelike appearance, and (iii) displaying undulating or serrated inner surfaces (*5–8*) (Fig. 1, C and D). Dendritic claws showed punctate expression of the postsynaptic protein PSD95 (Fig. 1D).

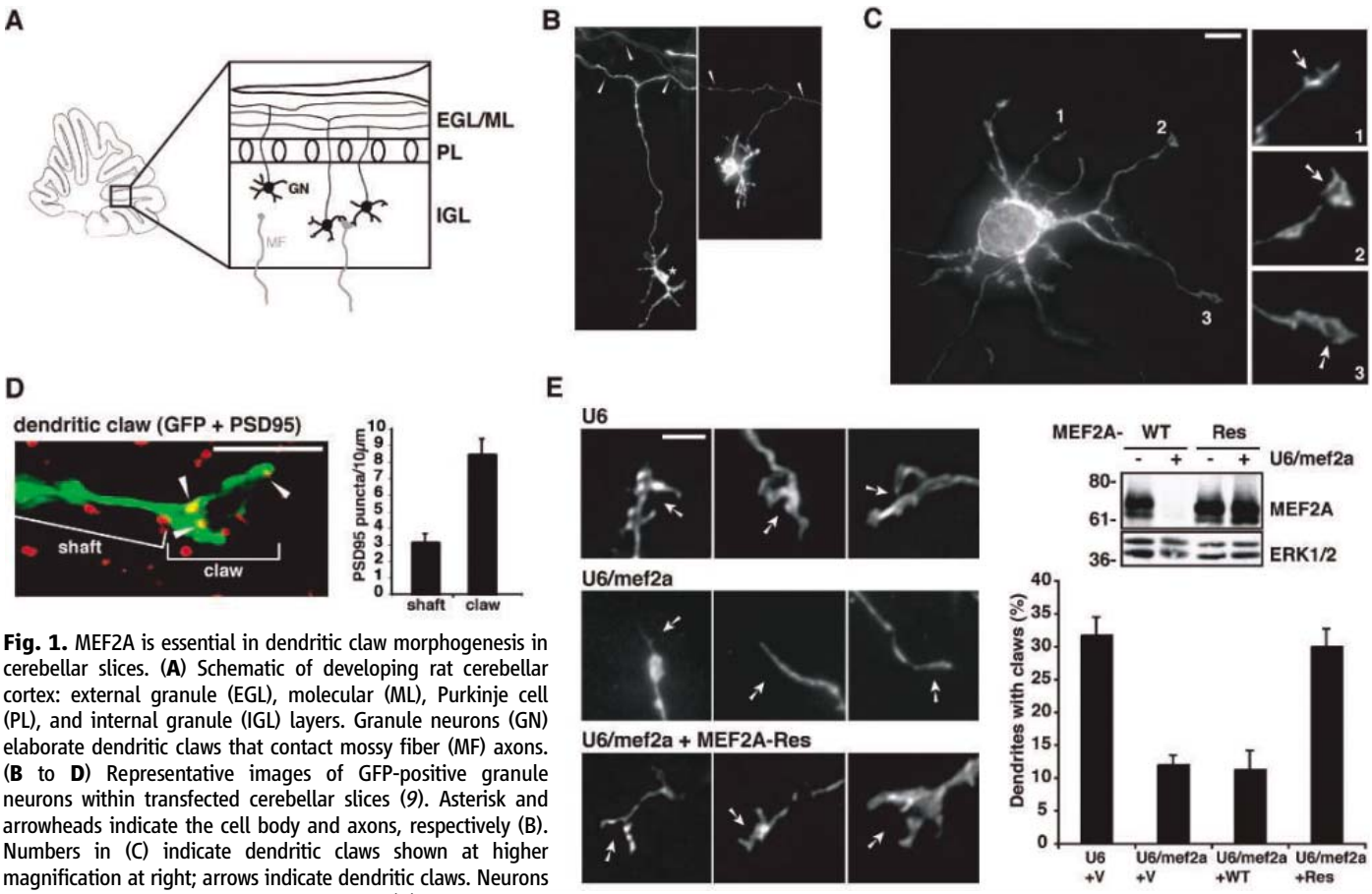

Fig. 1. MEF2A is essential in dendritic claw morphogenesis in cerebellar slices. (**A**) Schematic of developing rat cerebellar cortex: external granule (EGL), molecular (ML), Purkinje cell (PL), and internal granule (IGL) layers. Granule neurons (GN) elaborate dendritic claws that contact mossy fiber (MF) axons. (**B** to **D**) Representative images of GFP-positive granule neurons within transfected cerebellar slices (*9*). Asterisk and arrowheads indicate the cell body and axons, respectively (B). Numbers in (C) indicate dendritic claws shown at higher magnification at right; arrows indicate dendritic claws. Neurons are immunostained for GFP and PSD95 in (D). The claw and the shaft of the dendrite are indicated, and arrowheads indicate PSD95-positive puncta within the claw (*9*). PSD95 puncta density is significantly higher in the claws than in the shaft of dendrites ($P < 0.001$, *t* test; total neurons measured, $n = 22$) (*9*). (**E**) (Left) Cerebellar slices transfected with the control U6 or U6/mef2a plasmid, together with expression plasmids encoding MEF2A-WT or MEF2A-Res and GFP, and analyzed for dendritic claws (*9*). (Upper right) Lysates of 293T cells transfected with control U6 or U6/mef2a plasmid together with expression plasmids for MEF2A-WT or MEF2A-Res were immunoblotted for MEF2A (*9*). (Lower right) The number of dendritic claws was significantly reduced by MEF2A knockdown and in MEF2A-WT–expressing neurons but not in MEF2A-Res–expressing neurons in the presence of MEF2A knockdown, when compared with U6-transfected controls ($P < 0.001$, ANOVA followed by Bonferroni-Dunn post hoc test; total neurons measured, $n = 262$). Scale bars: 5 μm (C); 3 μm (D and E).

PSD95 puncta density was greater in the claw region than in the shaft of dendrites (Fig. 1D). Thus, granule neuron dendritic claws in cerebellar slices represent sites of postsynaptic differentiation.

We determined the effect of MEF2A knockdown induced by RNA interference (RNAi) on granule neuron dendritic morphogenesis. We transfected cerebellar slices with the U6/mef2a plasmid that encodes MEF2A hairpin RNAs ($MEF2A^{hpRNA}$) or the control U6 plasmid, together with a GFP expression plasmid (*9*). The $MEF2A^{hpRNA}$-expressing granule neurons had 60% fewer dendritic claws than control U6-transfected neurons, and their dendrites displayed tapered or bulbous tips instead of claws (Fig. 1E). In these dendrites, PSD95 puncta density was low in the tip region and no greater than in the shaft (fig. S2). The $MEF2A^{hpRNA}$-induced dendritic claw phenotype was not due to a reduction in dendritic growth (fig. S3). Together, these results suggest that MEF2A plays a key role in the morphogenesis of dendritic claws in the cerebellar cortex.

To exclude the possibility that the dendritic phenotype induced by MEF2A knockdown is the result of off-target effects of RNAi, we performed a rescue experiment. MEF2A RNAi induced the effective knockdown of MEF2A protein encoded by wild-type (WT) MEF2A cDNA but failed to reduce the expression of MEF2A encoded by an RNAi-resistant cDNA (MEF2A-Res) (Fig. 1E). In cerebellar slices, MEF2A-Res, but not MEF2A-WT, reversed the $MEF2A^{hpRNA}$-induced dendritic claw phenotype (Fig. 1E). Expression of MEF2A-Res induced dendritic claws of similar number, morphological appearance, and PSD95 density as those in control U6-transfected neurons (Fig. 1, D and E; fig. S2). These experiments indicate that the $MEF2A^{hpRNA}$-induced dendritic claw phenotype is the result of the specific knockdown of MEF2A.

To establish MEF2A function in dendritic claw development in vivo, we induced MEF2A knockdown in the postnatal cerebellum using electroporation-mediated gene transfer (*9*, *10*). We injected a control U6 or U6/mef2a plasmid that also encoded GFP into the cerebellar cortex of P3 rat pups and assessed dendritic claws in the cerebellum of these animals at P12. Granule neurons in control-transfected cerebella had PSD95-positive postsynaptic dendritic claws at the tips of their dendrites (Fig. 2, A and B). In addition, expression of the presynaptic protein synaptophysin was found juxtaposed to the surface of ~80% of dendritic claws (Fig. 2C). As in cerebellar slices, MEF2A knockdown reduced the number of dendritic claws in the cerebellum in vivo (Fig. 2D). Together, our findings suggest a physiological, cell-autonomous function for MEF2A in the mor-

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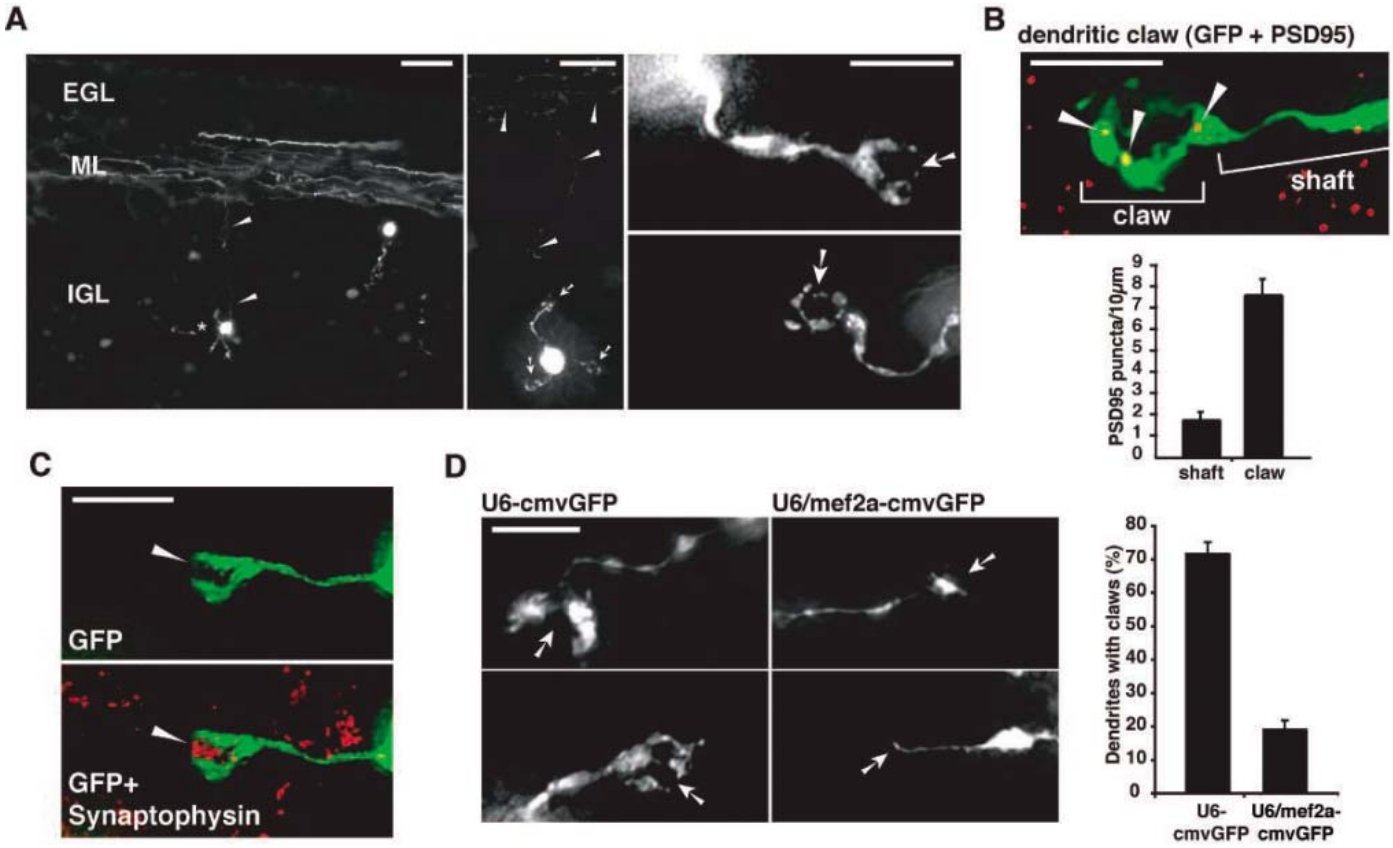

Fig. 2. MEF2A is required for postsynaptic dendritic differentiation in vivo. (**A**) The cerebellum of P3 rat pups was injected and electroporated with the U6-cmvGFP plasmid (*9*). Representative electroporated granule neurons with cell bodies in the IGL. Scale bars: 50 μm (left), 10 μm (middle), 5 μm (right). Asterisk, arrowheads, and arrows, respectively, indicate the cell body, parallel fibers, and dendritic claws of granule neurons. (**B** and **C**) Sections of U6-cmvGFP–electroporated cerebellum were immunostained with antibodies to GFP and PSD95 (B) or synaptophysin (C). An image of a dendritic claw analyzed as in Fig. 1D is shown, and PSD95 density is quantified below (B). PSD95 puncta density is significantly higher in the claw than in the shaft of dendrites ($P < 0.001$, *t* test; total neurons measured, $n = 13$). (**D**) Representative granule neuron dendrites from cerebella transfected with the control U6-cmvGFP or U6/mef2a-cmvGFP plasmid. Quantification indicates that MEF2A knockdown significantly reduces dendritic claw number in vivo ($P < 0.001$, *t* test; total neurons measured, $n = 124$). Scale bar, 5 μm (B to D).

phogenesis of dendritic claws in the developing cerebellar cortex.

Calcium signaling strongly influences the activity of MEF2s (*11*, *12*). Calcium entry through voltage-sensitive calcium channels (VSCCs) triggers MEF2 phosphorylation at distinct sites and calcineurin-mediated dephosphorylation at undetermined sites, which lead to enhanced MEF2-dependent transcription (*11*–*14*). Calcineurin has emerged as a critical regulator of dendritic spine morphology in hippocampal neurons (*15*). We reasoned that calcineurin might, therefore, control postsynaptic dendritic differentiation via a MEF2-regulated transcriptional mechanism.

We first determined the site of calcineurin-mediated dephosphorylation of MEF2A. Because calcineurin stimulates MEF2-dependent transcription, we reasoned that calcineurin might induce the dephosphorylation of MEF2A at Ser^{408}, whose phosphorylation inhibits MEF2-dependent transcription (*16*, *17*). Using antibodies that recognize MEF2A when phosphorylated on Ser^{408} (fig. S4) (*9*), we found that endogenous MEF2A was phosphorylated on Ser^{408} in neurons (Fig. 3A). When neurons were depolarized, MEF2A underwent rapid and robust dephosphorylation at Ser^{408}, an effect that was blocked in neurons treated with nimodipine, an inhibitor of L-type VSCCs, or cyclosporin A (CsA), an inhibitor of calcineurin (Fig. 3A; fig. S5). In human 293T embryonic kidney cells, MEF2A was constitutively phosphorylated at Ser^{408}, and coexpression of activated calcineurin induced dephosphorylation of MEF2A at this site (Fig. 3B). These results suggest that calcineurin mediates activity-induced dephosphorylation of MEF2A at Ser^{408} in neurons.

Intriguingly, Ser^{408} lies near a conserved SUMO (small ubiquitin-like modifier) acceptor site centered at Lys^{403} within a domain of MEF2A that represses transcription (*17*, *18*). Sumoylation of transcription factors typically induces transcriptional repression (*19*, *20*). MEF2 proteins can function as activators or repressors of transcription in a signal-dependent manner (*11*, *12*, *21*). We asked whether Ser^{408} dephosphorylation might regulate MEF2A sumoylation at Lys^{403} and MEF2's transcriptional repression function. First, we demonstrated that MEF2A Lys^{403} is modified by sumoylation in vitro and in cells (Fig. 3C; fig. S6). Interestingly, MEF2A was also acetylated in cells in a Lys^{403}-dependent manner (Fig. 3C).

To determine how dephosphorylation of Ser^{408} might regulate the Lys^{403} modifications, we expressed the MEF2A transactivation domain fused to the DNA binding domain of GAL4 (G4-MEF2A) together with a constitutively active form of calcineurin. Activated calcineurin inhibited sumoylation and enhanced acetylation of MEF2A in cells (Fig. 3D). A G4-MEF2A mutant in which Ser^{408} was replaced with alanine (G4-MEF2AS408A) had reduced sumoylation and enhanced acetylation as compared with G4-MEF2A (Fig. 3E). Expression of the SUMO E2 ligase Ubc9 in cells increased sumoylation and inhibited the acetylation of G4-MEF2A, but not of G4-MEF2AS408A (fig. S7). Together, these results suggest that the calcineurin-induced dephosphorylation of MEF2A at Ser^{408} promotes a sumoylation to acetylation switch at Lys^{403}.

In granule neurons, endogenous sumoylated MEF2A was detected as an *N*-ethylmaleimide (NEM)–sensitive MEF2 immunoreactive band

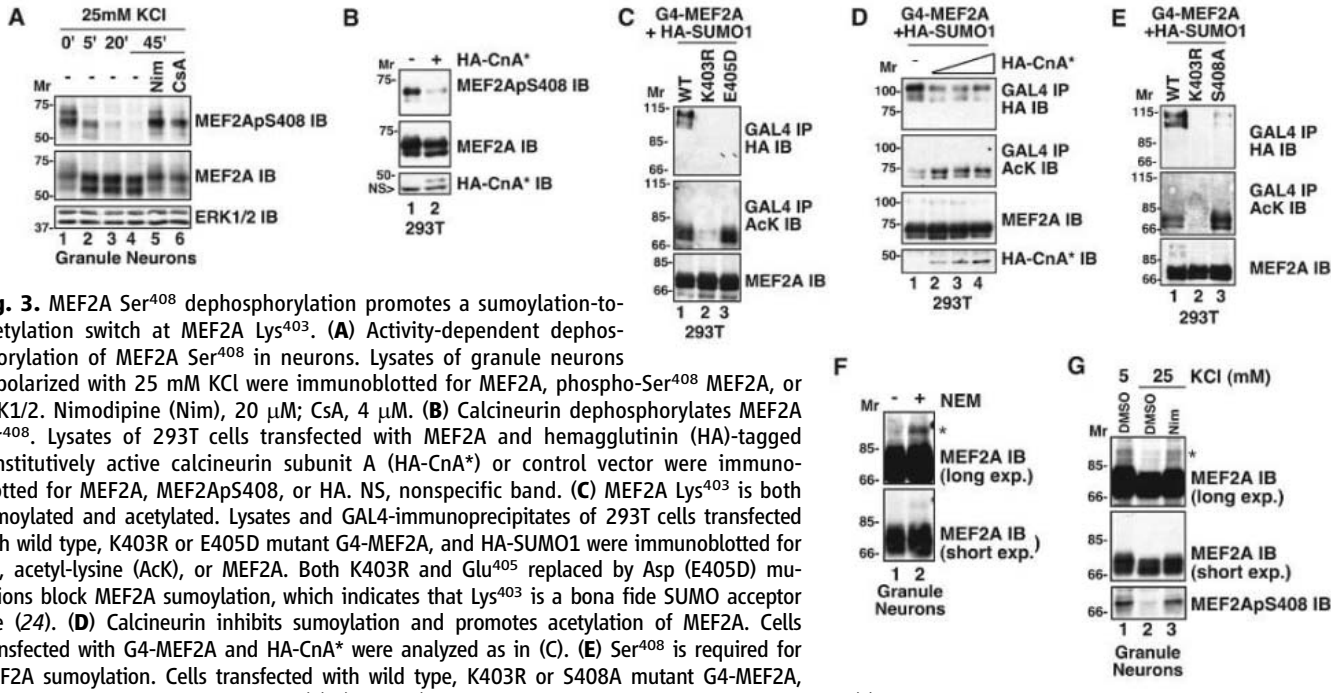

Fig. 3. MEF2A Ser[408] dephosphorylation promotes a sumoylation-to-acetylation switch at MEF2A Lys[403]. (**A**) Activity-dependent dephosphorylation of MEF2A Ser[408] in neurons. Lysates of granule neurons depolarized with 25 mM KCl were immunoblotted for MEF2A, phospho-Ser[408] MEF2A, or ERK1/2. Nimodipine (Nim), 20 μM; CsA, 4 μM. (**B**) Calcineurin dephosphorylates MEF2A Ser[408]. Lysates of 293T cells transfected with MEF2A and hemagglutinin (HA)-tagged constitutively active calcineurin subunit A (HA-CnA*) or control vector were immunoblotted for MEF2A, MEF2ApS408, or HA. NS, nonspecific band. (**C**) MEF2A Lys[403] is both sumoylated and acetylated. Lysates and GAL4-immunoprecipitates of 293T cells transfected with wild type, K403R or E405D mutant G4-MEF2A, and HA-SUMO1 were immunoblotted for HA, acetyl-lysine (AcK), or MEF2A. Both K403R and Glu[405] replaced by Asp (E405D) mutations block MEF2A sumoylation, which indicates that Lys[403] is a bona fide SUMO acceptor site (*24*). (**D**) Calcineurin inhibits sumoylation and promotes acetylation of MEF2A. Cells transfected with G4-MEF2A and HA-CnA* were analyzed as in (C). (**E**) Ser[408] is required for MEF2A sumoylation. Cells transfected with wild type, K403R or S408A mutant G4-MEF2A, and HA-SUMO1 were analyzed as in (C). (**F** and **G**) Endogenous MEF2A is sumoylated in neurons. (F) Granule neurons in nondepolarizing concentrations of KCl (5 mM) were lysed in the presence or absence of the isopeptidase inhibitor NEM and immunoblotted for MEF2A. Asterisk indicates a form of MEF2A of appropriate size for sumoylated MEF2A (fig. S8). (G) Granule neurons in media containing nondepolarizing (5 mM) or depolarizing (25 mM) concentrations of KCl in the presence of Nim or its control vehicle dimethyl sulfoxide (DMSO) were lysed in the presence of NEM and immunoblotted as in (F). MEF2A is also acetylated in neurons in a VSCC- and calcineurin-dependent manner (fig. S9).

of appropriate molecular size by immunoblotting with antibodies to MEF2A (Fig. 3F; fig. S8). Membrane depolarization of neurons led to an almost complete reduction of sumoylated MEF2A, an effect that tightly correlated with Ser[408] dephosphorylation (Fig. 3G). Endogenous MEF2A was acetylated in depolarized neurons (fig. S9). Incubation of depolarized neurons with the VSCC inhibitor nimodipine or the calcineurin inhibitor CsA increased sumoylation and decreased acetylation of endogenous MEF2A (Fig. 3G; fig. S9).

We assessed the consequences of Ser[408] dephosphorylation-induced Lys[403] modifications of MEF2A on transcription. Replacement of Ser[408] with alanine (S408A) or Lys[403] with arginine (K403R) in G4-MEF2A similarly enhanced transcription in neurons or 293T cells (Fig. 4A; fig. S10). The S408A and K403R mutants of MEF2A are both deficient in sumoylation, yet the S408A mutant enhances MEF2A acetylation (Fig. 3E). In view of these results, the phenocopy of the S408A and K403R mutants in the reporter assay supports the conclusion that sumoylation is the critical modification of Lys[403], leading to repression of transcription. Acetylation of Lys[403] may thus serve to prevent sumoylation of MEF2A.

Fusion of a SUMO moiety to transcription factors mimics the effect of SUMO that is covalently linked to proteins on the native lysine (*22*). A MEF2A-SUMO fusion protein potently inhibited the ability of coexpressed wild-type MEF2A to induce a MEF2-responsive [MEF2 response element (MRE)–dependent] reporter gene in cells (Fig. 4B). Sumoylation did not appear to alter MEF2A's subnuclear localization or stability (fig. S11). Together, our findings suggest that Lys[403]-sumoylated MEF2A represses transcription and that Ser[408] dephosphorylation inhibits Lys[403] sumoylation and, thereby, derepresses MEF2A-induced transcription.

To characterize the role of the calcium-MEF2A signaling pathway in dendritic claw morphogenesis in the cerebellar cortex, we first tested the effect of MEF2A sumoylation on dendritic claw differentiation. Expression of MEF2A-SUMO increased the number of dendritic claws compared with MEF2A-expressing or control-transfected neurons (Fig. 4C), which suggested that a transcriptional repressor form of MEF2A stimulates dendritic claw differentiation. In support of this conclusion, expression of a protein in which the MADS/MEF2 domains were fused to the transcriptional repressor Engrailed (MEF2-EN), which potently repressed MRE-dependent transcription, led to an increase in the number of dendritic claws in cerebellar slices (fig. S12).

We next determined the effect of the endogenous calcium-induced cascade of modifications at Ser[408] and Lys[403] of MEF2A on dendritic claw differentiation. Incubation of cerebellar slices with nimodipine or CsA increased dendritic claw number (Fig. 4D), which suggested that VSCC or calcineurin activation inhibits dendritic claw development. We then tested the ability of the S408A or K403R mutant of MEF2A-Res to rescue the MEF2A^{hpRNA}-induced dendritic claw phenotype in cerebellar slices. The S408A mutant mimics calcineurin-induced dephosphorylation of MEF2A Ser[408], whereas both S408A and K403R mutants of MEF2A are deficient in sumoylation and transcriptional repression (Figs. 3 and 4A). In contrast to MEF2A-Res, neither mutant of MEF2A-Res reversed MEF2A^{hpRNA}-inhibition of dendritic claw morphogenesis (Fig. 4E). Fusion of SUMO with the S408A mutant of MEF2A-Res protein gave this protein the ability to induce dendritic claw differentiation in the presence of MEF2A knockdown (Fig. 4F). Thus, the rescue experiments suggest that an endogenously sumoylated transcriptional repressor form of MEF2A promotes dendritic claw differentiation. Together, our results also suggest that the calcium-induced Ser[408] dephosphorylation and consequent inhibition of Lys[403] sumoylation of MEF2A suppress dendritic claw morphogenesis (see model in fig. S13).

We investigated the mechanism by which sumoylated MEF2A promotes dendritic claw differentiation. Transcription of the gene encoding the transcription factor Nur77 is induced by a calcineurin-MEF2 signaling pathway in immune cells (*23*). Endogenous MEF2A was found to occupy the endogenous Nur77 promoter in granule neurons (Fig. 5A). Nur77 mRNA abundance and Nur77 promoter–mediated transcription increased in depolarized neurons in a

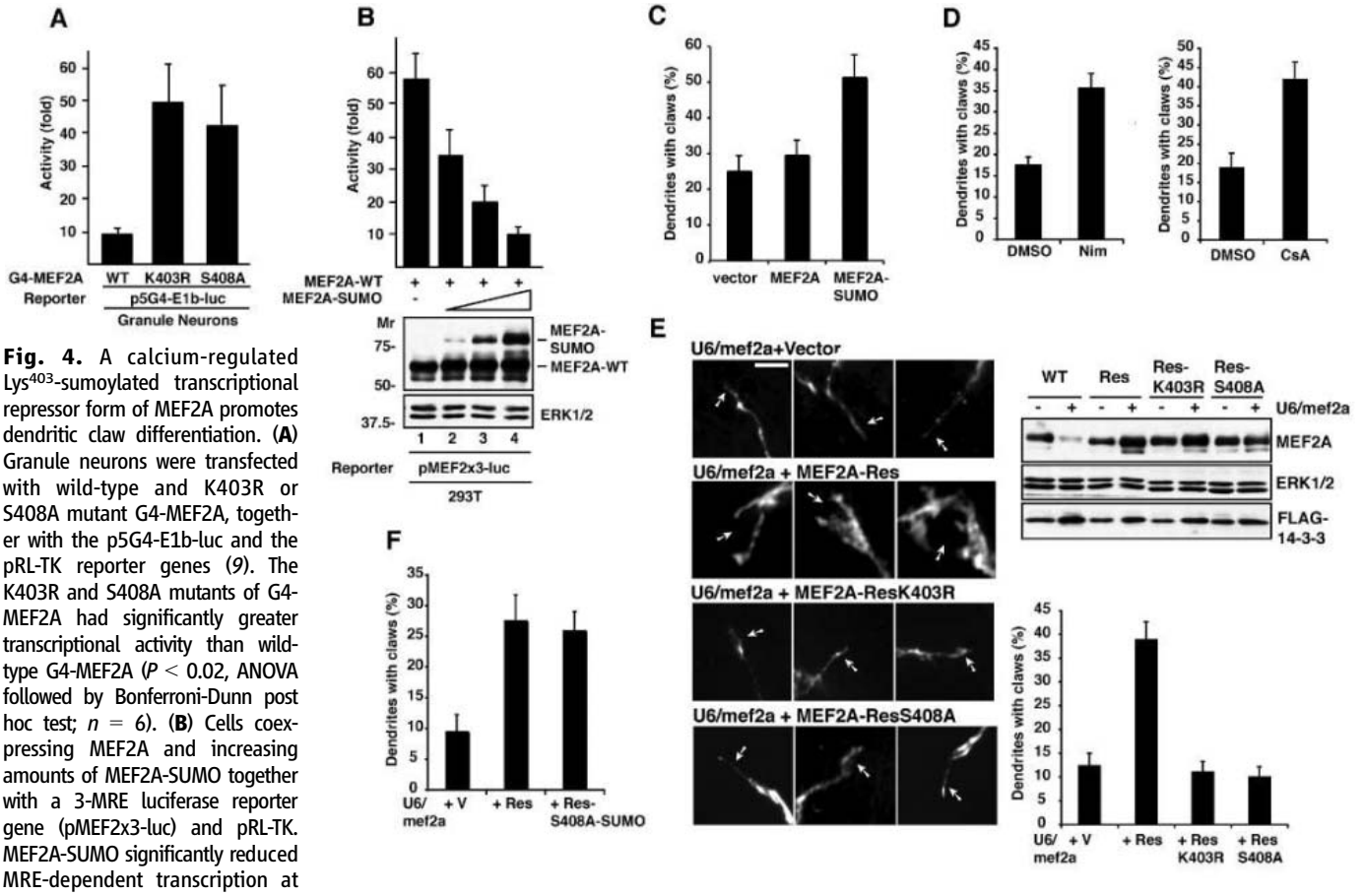

Fig. 4. A calcium-regulated Lys^{403}-sumoylated transcriptional repressor form of MEF2A promotes dendritic claw differentiation. (**A**) Granule neurons were transfected with wild-type and K403R or S408A mutant G4-MEF2A, together with the p5G4-E1b-luc and the pRL-TK reporter genes (*9*). The K403R and S408A mutants of G4-MEF2A had significantly greater transcriptional activity than wild-type G4-MEF2A ($P < 0.02$, ANOVA followed by Bonferroni-Dunn post hoc test; $n = 6$). (**B**) Cells coexpressing MEF2A and increasing amounts of MEF2A-SUMO together with a 3-MRE luciferase reporter gene (pMEF2x3-luc) and pRL-TK. MEF2A-SUMO significantly reduced MRE-dependent transcription at all amounts tested ($P < 0.01$, ANOVA followed by Bonferroni-Dunn post hoc test; $n = 5$) (*9*). Lysates were also immunoblotted for MEF2 or extracellular signal–regulated kinase types 1 and 2 (ERK1/2). (**C**) Cerebellar slices were transfected with control vector, MEF2A, or MEF2A-SUMO. Dendritic claw number was significantly increased by MEF2A-SUMO when compared with MEF2A-expressing or vector-transfected neurons ($P < 0.005$, ANOVA followed by Bonferroni-Dunn post hoc test; total neurons measured, $n = 53$) (*9*). (**D**) Cerebellar slices transfected as in Fig. 1C were treated with Nim (20μM), CsA (4μM), or vehicle (DMSO). Dendritic claw number was significantly increased by Nim or CsA as compared with vehicle ($P < 0.001$, ANOVA followed by Bonferroni-Dunn post hoc test; total neurons measured, $n = 162$) (*9*). (**E**) (Left) Cerebellar slices transfected with the U6/mef2a plasmid together with MEF2A-Res or with K403R or S408A mutants of MEF2A-Res were analyzed as in Fig. 1E. Scale bar, 3 μm. The number of claws was significantly higher in granule neurons that expressed MEF2A-Res but not MEF2A-ResK403R or MEF2A-ResS408A in the presence of MEF2A knockdown when compared with MEF2A knockdown alone ($P < 0.001$, ANOVA followed by Bonferroni-Dunn post hoc test; total neurons measured, $n = 133$). (Upper right) Lysates of 293T cells transfected with the control or U6/mef2a plasmid together with MEF2A-WT, MEF2A-Res, K403R or S408A mutant of MEF2A-Res, and FLAG-14-3-3 were immunoblotted with the indicated antibodies. (**F**) Cerebellar slices transfected with the U6/mef2a plasmid, together with MEF2A-Res or MEF2A-ResS408A-SUMO. The number of claws was significantly higher in neurons expressing MEFA-Res or MEF2A-ResS408A-SUMO in the presence of MEF2A knockdown when compared with MEF2A knockdown alone ($P < 0.005$, ANOVA followed by Bonferroni-Dunn post hoc test; $n = 105$).

VSSC- and calcineurin-dependent manner (Fig. 5, B and C). Both MEF2A-SUMO and MEF2-EN inhibited depolarization-induced Nur77 transcription (Fig. 5D). In cerebellar slices, expression of a dominant interfering form of Nur77 increased the number of dendritic claws (Fig. 5E). Thus, Nur77 represents a MEF2A target gene whose repression by sumoylated MEF2A contributes to dendritic claw differentiation (fig. S13).

We have discovered a transcriptional mechanism that may orchestrate postsynaptic dendritic development in the mammalian brain. Our findings indicate that the transcription factor MEF2A plays a key role in the morphogenesis of granule neuron dendritic claws in the cerebellar cortex. The modifications of MEF2A required for postsynaptic differentiation occur within a phosphorylation-regulated sumoylation-acetylation switch (SAS) peptide motif that is conserved in all major MEF2 isoforms except MEF2B, as well as in several other transcription factor families (table S1). Thus, a phosphorylation-dependent switch between sumoylation and acetylation in transcription factors may play a widespread role in signal-regulated transcription and regulate diverse biological processes, including synapse development and plasticity.

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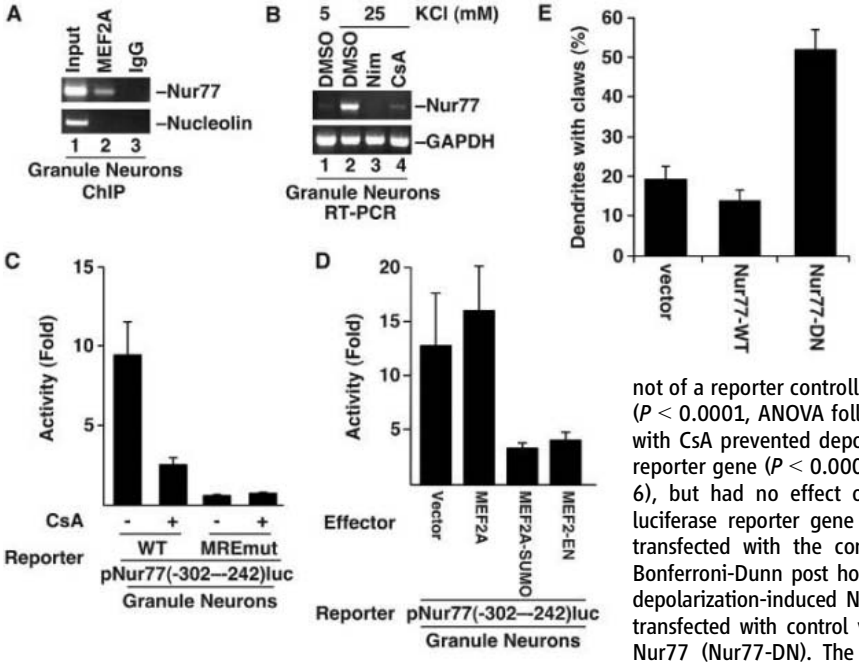

Fig. 5. Nur77 repression by MEF2A-SUMO contributes to dendritic claw morphogenesis. (**A**) Endogenous MEF2A is associated with the endogenous Nur77 promoter but not nucleolin (control) in granule neurons as determined by chromatin immunoprecipitation analysis. (**B**) Depolarization induces Nur77 gene expression in neurons in a VSCC- and calcineurin-dependent manner. RNA of granule neurons treated for 1 hour in the presence or absence of 25 mM KCl and vehicle (DMSO), Nim, or CsA was subjected to reverse transcription polymerase chain reaction (RT-PCR) by using primers specific to Nur77 or GAPDH. (**C**) Depolarization of granule neurons significantly induced expression of a luciferase reporter gene controlled by a Nur77 promoter containing WT MRE but not of a reporter controlled by a Nur77 promoter containing mutant MRE (MREmut) ($P < 0.0001$, ANOVA followed by Bonferroni-Dunn post hoc test; $n = 6$). Treatment with CsA prevented depolarization-induced expression of the WT Nur77–luciferase reporter gene ($P < 0.0001$, ANOVA followed by Bonferroni-Dunn post hoc test; $n = 6$), but had no effect on MREmut Nur77–luciferase reporter gene. (**D**) Nur77–luciferase reporter gene activity was significantly induced in depolarized neurons transfected with the control vector or MEF2A ($P < 0.005$, ANOVA followed by Bonferroni-Dunn post hoc test; $n = 4$). Both MEF2A-SUMO and MEF2-EN repressed depolarization-induced Nur77–luciferase reporter activity. (**E**) Cerebellar slices were transfected with control vector, wild-type Nur77 (Nur77-WT), or dominant-negative Nur77 (Nur77-DN). The number of claws was significantly increased in neurons expressing Nur77-DN compared with control-transfected neurons or neurons expressing Nur77-WT ($P < 0.001$, ANOVA followed by Bonferroni-Dunn post hoc test; total neurons measured, $n = 114$).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5763/1012/DC1
Materials and Methods
Figs. S1 to S13
Table S1
References and Notes

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Role of Noradrenergic Signaling by the Nucleus Tractus Solitarius in Mediating Opiate Reward

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Norepinephrine (NE) is widely implicated in opiate withdrawal, but much less is known about its role in opiate-induced locomotion and reward. In mice lacking dopamine β-hydroxylase (DBH), an enzyme critical for NE synthesis, we found that NE was necessary for morphine-induced conditioned place preference (CPP; a measure of reward) and locomotion. These deficits were rescued by systemic NE restoration. Viral restoration of DBH expression in the nucleus tractus solitarius, but not in the locus coeruleus, restored CPP for morphine. Morphine-induced locomotion was partially restored by DBH expression in either brain region. These data suggest that NE signaling by the nucleus tractus solitarius is necessary for morphine reward.

The pleasurable experiences associated with taking a drug encourage repeated usage, which can lead to neurochemical changes that promote addiction. Seminal studies of the neurotransmitters underlying opiate reward implicated the catecholamines NE and dopamine (DA), but the role of DA in this process has received the most attention (*1*, *2*). Although many studies implicate NE in the adverse effects of opiate withdrawal (*3*), its role in mediating the rewarding and stimulatory effects of opiates remains equivocal. We examined opiate-mediated reward and locomotion in dopamine β-hydroxylase knockout (DBH-KO) mice, which cannot synthesize NE (*4*, *5*). This genetic lesion is specific and complete, but it is conditional in that NE synthesis can be restored by administration of a pseudo-substrate (*4*).

To assess opiate reward in DBH-KO mice, we used a balanced and unbiased conditioned place preference (CPP) paradigm (*6*). Rodents can learn to associate the pleasurable aspects of a stimulus with the environments in which they experience that stimulus, and they preferentially seek such environments (referred to as CPP) while avoiding environments where unpleasant stimuli are encountered (conditioned place aversion, CPA). Quantification of time spent in a drug-paired environment after conditioning can be used as a measure of the rewarding properties of that drug (*6*). DBH-KO mice showed no preference for the morphine-paired environment at any dose tested, but they showed aversion to the drug-paired side at a dose of 25 mg/kg intraperitoneally (ip) (Fig.

1B). In contrast, littermate controls showed a typical CPP to morphine at doses of 15, 20, and 25 mg/kg (Fig. 1A). Noradrenergic neurotransmission was restored in DBH-KO mice by administering L-3,4-dihydroxyphenylserine (DOPS), which can be converted to NE in the absence of DBH (*4*). Treatment with DOPS rescued CPP for morphine in DBH-KO mice but did not affect control mice (Fig. 1, A and B). To ensure that this deficit in CPP was due to lack of morphine reward rather than a learning or attention deficit, we assessed the ability of DBH-KO mice to form a CPP for food; DBH-KO mice showed a robust preference for a food-paired environment (Fig. 1C). Other studies have shown that DBH-KO mice can form a CPP to cocaine (*7*).

Acute administration of morphine also stimulates locomotion in rodents (*8*–*11*). At all doses tested, morphine increased locomotion in control mice; however, DBH-KO mice were significantly less active than controls at each dose of morphine, but not with saline (Fig. 1D). DOPS administration before morphine (20 mg/kg) restored morphine-induced locomotion in DBH-KO mice but did not affect control mice (Fig. 1D).

Two noradrenergic nuclei, the locus coeruleus (LC) and the nucleus tractus solitarius (NTS), have been implicated in the aversive effects of opiate withdrawal (*3*, *12*). We hypothesized that they may also play a role in the rewarding properties of opiates. Therefore, we selectively restored DBH expression in either the LC or the NTS of DBH-KO mice with the use of adeno-associated virus (AAV) encoding DBH and the fluorescent reporter protein DsRed2 (AAV1-DBH-DsRed2, Fig. 2A); we also used a control virus expressing luciferase (AAV1-Luc). Bilateral injection of AAV into the NTS or LC resulted in DsRed expression in the respective nuclei (Fig. 2, B and C) but not in other noradrenergic cell groups (*13*). Most of the DsRed-positive neurons were noradrenergic (but in the NTS, also possibly adrenergic), as indicated by costaining with antibodies to tyrosine hydroxylase (TH) (Fig. 2, D and E). After AAV1-DBH-DsRed2 injection into the LC, expression of DBH was observed in neuronal processes in the anterior cingulate area of the prefrontal cortex (PFC), a brain region that receives noradrenergic innervation solely from the LC (*14*) (Fig. 2F). When AAV1-DBH-DsRed2 was injected into the NTS, DBH expression was not observed in the PFC (Fig. 2G), but it was found in neuronal processes in the nucleus accumbens (NAc) (Fig. 2I) and the bed nucleus of the stria terminalis (BNST, fig. S1), which receive the majority of noradrenergic input from the NTS rather than from the LC (*12*, *14*, *15*). These latter areas were sparsely labeled after AAV1-DBH-DsRed2 transduction of the LC (Fig. 2H).

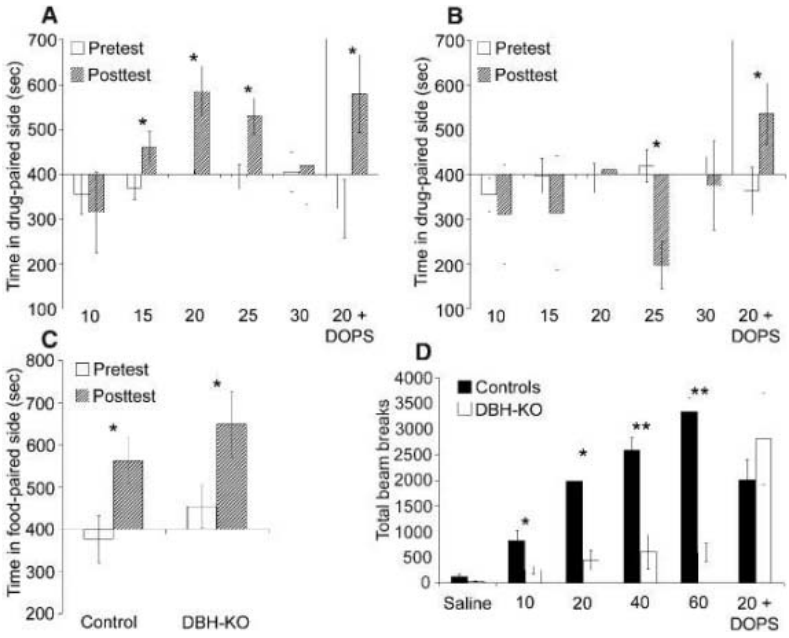

Fig. 1. CPP and locomotor responses of DBH-KO and control mice to morphine. (**A**) Control mice treated with various doses of morphine during the conditioning phase develop CPP at intermediate doses (mg/kg ip); administration of DOPS had no effect (n = 7 to 15 mice per group). (**B**) DBH-KO mice treated with various doses of morphine during the conditioning phase of the CPP paradigm fail to manifest CPP; administration of DOPS before the conditioning with morphine at 20 mg/kg restored CPP (n = 7 to 13 mice per group). Analysis revealed a significant genotype effect but no genotype × dose interaction. (**C**) Both DBH-KO and control mice form a CPP for food (n = 6 or 7 mice per group). (**D**) Control mice show increased locomotion in response to all morphine doses tested, whereas the response of DBH-KO mice is significantly blunted (n = 6 to 10 mice per group). CPP data are expressed as means ± SEM of time spent on the drug-paired side before and after conditioning. Locomotor data are expressed as average total beam breaks ± SEM during a 2-hour session. *P < 0.05, **P < 0.01.

Expression of DBH in selected noradrenergic nuclei restored behavioral responses to morphine. Bilateral injection of AAV1-DBH-DsRed2 into the NTS completely rescued CPP for morphine, whereas injection of AAV1-Luc had no effect (Fig. 3A). In contrast, injections of either AAV1-DBH-DsRed2 or AAV1-Luc into the LC failed to rescue CPP for morphine (Fig. 3B). Injections of AAV1-DBH-DsRed2 into either the LC or the NTS partially restored morphine-induced locomotion, whereas injections of AAV1-Luc into either region had no effect (Fig. 3C). Viral injections into either the LC or the NTS had no effect on locomotor response to saline injection (fig. S2).

To explore how opiates affect NTS neurons, we measured cFos activation in the NTS of control mice given a dose of morphine (20 mg/kg) that induces optimal CPP. We observed a significant increase in cFos labeling of both TH-positive and TH-negative NTS neurons relative to saline-injected controls, as previously documented in rats (*16*) (Fig. 3D) (fig. S3).

We conclude that the inability of DBH-KO mice to form a normal CPP to morphine is due to an inability to experience morphine reward, not to an impairment of their ability to learn a place association or to a general deficit in reward. These mice also manifest a blunted locomotor response to morphine. Neither of these deficits is due to chronic compensatory changes in response to loss of NE, because acute restoration of NE via administration of DOPS rescues these phenotypes. Our data suggest that NTS neurons are activated by morphine and that NE signaling by this nucleus is critical to opiate reward, whereas NE release from both the LC and the NTS contributes to morphine-induced locomotion.

A role for NE in opiate reward was suggested three decades ago (*2*, *16*), in part because inhibition of DBH activity attenuated opiate reward (*1*). However, subsequent studies of the LC yielded mixed results, and the role of other noradrenergic cell groups was not studied (*16*). Some recent studies have implicated NE in opiate reward, but the results are hard to

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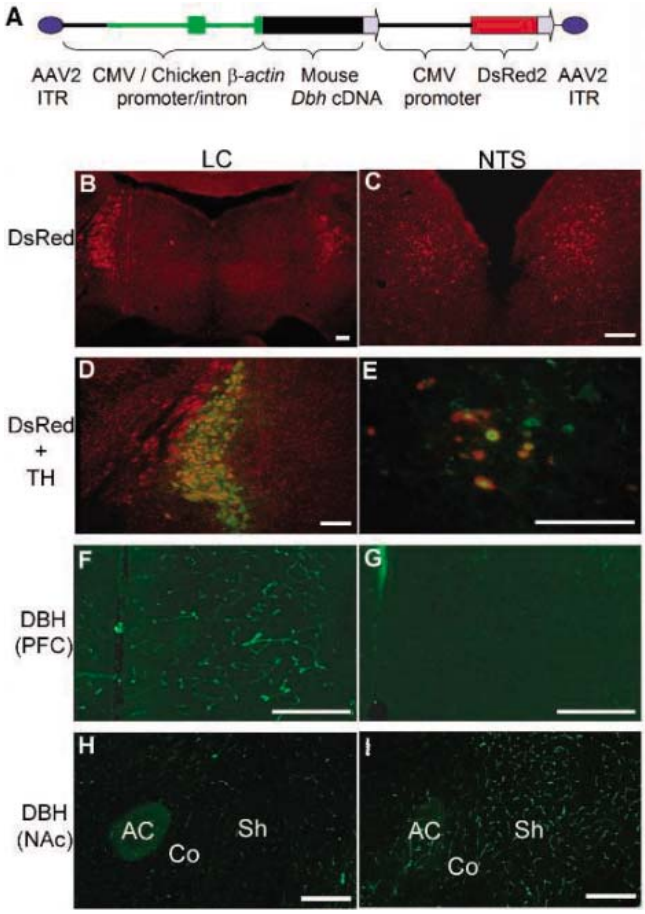

Fig. 2. Restoration of DBH expression in selected noradrenergic nuclei by viral transduction. (**A**) Schematic of AAV1-DBH-DsRed2 construct that was used to make AAV for injection. (**B**) Representative coronal section through the brain of an animal injected with AAV1-DBH-DsRed2 bilaterally into the LC. Red cells are neurons expressing the DsRed transgene. (**D**) The same section labeled with an antibody to TH, a marker of noradrenergic neurons. (**C** and **E**) Representative sections through the brain of an animal injected with AAV1-DBH-DsRed2 bilaterally into the NTS with visualization of DsRed (C) and DsRed and TH (E). (**F** to **I**) Immunohistochemistry with antibodies to DBH in PFC (anterior cingulate region) (F) or shell of NAc (H) after injection of AAV1-DBH-DsRed2 into the LC; similar sections and staining of PFC (G) and NAc (I) after injection of AAV1-DBH-DsRed2 into the NTS. AC, anterior commissure; Co, NAc core; Sh, NAc shell. Scale bars, 100 μm.

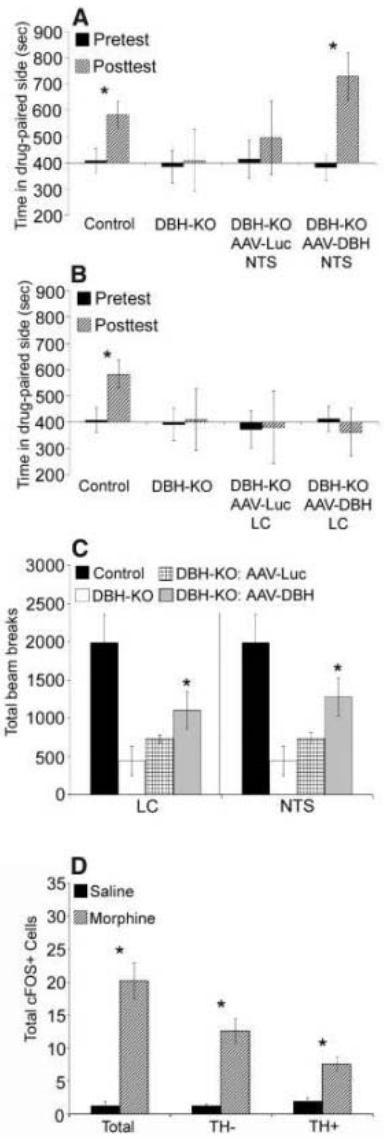

Fig. 3. Behavioral consequences of restoring DBH expression to the NTS or LC. (**A**) Injection of AAV1-DBH-DsRed2 into the NTS of DBH-KO mice restored morphine CPP, whereas AAV1-Luc did not (n = 7 to 13 mice per group). (**B**) Injection of AAV1-DBH-DsRed2 into the LC of DBH-KO mice did not restore CPP for morphine, and neither did AAV1-Luc (n = 7 to 13 mice per group). (**C**) Injection of AAV1-DBH-DsRed2 into either the NTS or the LC of DBH-KO mice partially rescued morphine-induced locomotion, whereas AAV1-Luc had no effect (n = 5 to 11 mice per group). (**D**) Morphine administration (20 mg/kg) increased the number of cFos-positive cells in the NTS, including those that are TH-positive and TH-negative (n = 4 or 5 mice per group). *P < 0.05.

interpret. Adrenergic receptor agonists and antagonists have been shown to alter CPP for morphine (*17*–*19*); however, the results are conflicting (*17*–*19*) and they are confounded by the fact that some noradrenergic drugs induce CPP or CPA on their own (*6*, *17*) or are not selective for adrenergic receptors (*20*). The finding that adrenergic receptor (*Adra1b*)–null mice have attenuated CPP at one dose of morphine supports our results (*10*); however, these mice have other learning deficits and compensatory changes that confound interpretation of their inability to establish CPP for morphine (*21*–*23*). A recent study showed that 6-hydroxydopamine lesions of noradrenergic fibers in the PFC, which arise from the LC (*14*), attenuate morphine CPP (*8*). The apparent discrepancy with our findings may be explained by differences in technique: Ablation of noradrenergic fibers in the PFC would eliminate not only NE signaling but also that of cotransmitters released from LC neurons. In addition, disruption of noradrenergic signaling in the PFC could lead to deficits in attention or memory (*24*) that would impair an animal's ability to learn a place association without affecting other forms of memory, thereby disrupting morphine CPP without affecting opiate reward. Because the LC and NTS are extensively interconnected, it is also possible that interactions between these two nuclei regulate morphine CPP in normal mice.

How noradrenergic neurotransmission from the NTS regulates opiate reward remains to be explored. Our cFos data and that of others (*25*) suggest that morphine increases NE release, and possibly epinephrine release, by NTS neurons. The extended amygdala (EA) is extensively innervated by the NTS (*12*, *14*, *15*), and it has been implicated in the aversive properties of opiate withdrawal and in opiate-seeking behaviors (*12*, *26*, *27*). It has been proposed that NE derived from the NTS, rather than the LC, mediates these effects (*12*, *28*), which raises the possibility that NE signaling within the EA may also mediate the rewarding aspects of morphine. Which adrenergic receptors are involved and which neuronal circuits are affected by NE signaling from the NTS remain to be identified.

The partial rescue of morphine-induced locomotion that we observed after AAV injection into the LC is probably due to NE release in the PFC, where it is known to regulate opiate-induced locomotion (*9*). However, our results indicate that NE release from the NTS also contributes to locomotion. Both the LC and the NTS project to the ventral tegmental area, where NE may regulate DA neuron activity (*14*, *29*) and thereby facilitate locomotion and reward (*6*).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S3

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Causal Reasoning in Rats

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Empirical research with nonhuman primates appears to support the view that causal reasoning is a key cognitive faculty that divides humans from animals. The claim is that animals approximate causal learning using associative processes. The present results cast doubt on that conclusion. Rats made causal inferences in a basic task that taps into core features of causal reasoning without requiring complex physical knowledge. They derived predictions of the outcomes of interventions after passive observational learning of different kinds of causal models. These competencies cannot be explained by current associative theories but are consistent with causal Bayes net theories.

The ability to acquire and reason with causal knowledge is among our most central human cognitive competences (*1*). Causal knowledge serves two important functions: It allows us to predict outcomes on the basis of observations, and it underlies our ability to control events in the world. We investigated whether animals understand the relation between observations and interventions, which some philosophers regard as a core feature of causal reasoning (*2–4*).

Although a number of psychologists have claimed that both humans and animals use basic associative mechanisms to learn about causal relations (*5*), human studies have demonstrated a deeper understanding of causal relations that cannot be reduced to associative learning (*6–8*). In contrast, research on the cognitive competencies of nonhuman primates concludes that they demonstrate a superficial understanding of the association between tool use and its effects but fail to comprehend the unobservable physical mechanisms underlying these relations [(*9–11*), but see (*12*, *13*)]. It may well be, however, that nonhuman animals lack knowledge about physical mechanisms but still are capable of basic causal reasoning. The capacity to derive predictions for interventions after purely observational learning is a core competency that is not reducible to associative learning (*14*).

Humans and animals can learn associations between passively observed events (Pavlovian conditioning) as well as between interventions and outcomes (instrumental conditioning). Moreover, these two learning modes may interact (*15*). An understanding of the interrelations between observations ("seeing") and interventions ("doing"), however, requires more sophisticated representations. Simple transfer from observational learning can lead to inadequate predictions for interventions. For example, barometer readings statistically predict the weather, but at the same time, setting the barometer to an arbitrary reading does not influence the weather. Both relations could be learned with associative mechanisms in separate observational and instrumental learning trials, but associative theories are incapable of deriving correct predictions for interventions after observational learning when no prior instrumental learning is available.

The causal model in Fig. 1A shows how predictions for interventions can be derived from observations. Imagine that an animal learns in an observational Pavlovian learning phase that a light cue (L) temporally precedes both a tone stimulus (T) and food (F), thus learning a common-cause model with two effects (top panel). After learning this model, observing T should, via L, lead to the predictive inference that F should also be present. However, if the animal learns in the test phase that a newly introduced lever turns on T, it should be more

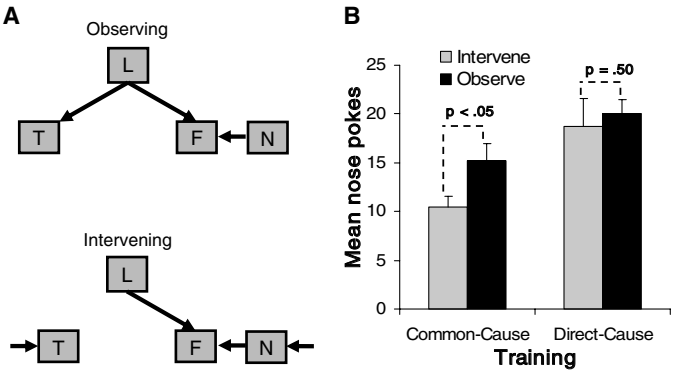

Fig. 1. (**A**) Causal model used in experiment 1. L (light) is the common cause of T (tone) and F (food); N (noise) is the direct cause of F. (Top panel) Observed causal relations. (Bottom panel) Model modified under the assumption of an intervention in T and N. (**B**) Experiment 1: Mean nose pokes in response to test stimulus T ($P < 0.05$) in the common-cause condition and to N ($P > 0.50$) in the direct-cause condition after a lever press (intervene) or no lever press (observe). Bars indicate SEM. Planned comparisons from a two-way mixed analysis of variance (ANOVA) are shown. There was a main effect of causal model (common or direct), $F(1, 21) = 6.01$, $P < 0.05$, and an interaction between causal model and test condition (intervene or observe), $F(1, 21) = 4.31$, $P = 0.05$.

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reluctant to predict F (bottom panel). Generating T by means of an alternative cause—the lever—does not predict F because the manipulation of an effect does not influence its cause (L). A dissociation between seeing and doing would be remarkable, because in the observational learning phase T is positively correlated with L.

The only theoretical model that derives correct predictions for interventions from observational learning data is causal Bayes nets (*2*–*4*). Predictions for observations make use of the full causal model acquired during observational learning (top panel). Predictions for interventions, however, are based on a modified graph (bottom panel); the insight that generating T in the common-cause model happens independently of its usual cause L is modeled by removing the causal arrow that leads into the manipulated effect: a manipulation called graph surgery (*3*). Because the manipulated T is unrelated to L, the likelihood of L's other effect F should not be altered by T's presence.

A possible alternative associationist explanation of the failure to expect F after an intervention in T may be that the animal does not expect F because it lacks prior instrumental learning experiences relating lever presses to F. This alternative theory, however, erroneously also predicts a failure to expect F in the presence of noise (N), after these events had been paired during observational learning (Fig. 1A). Because of the direct causal link between N and F, causal Bayes nets predict that animals should equally expect F, regardless of whether N is observed or generated by an intervention. Recent research with similar tasks has shown (*14*) that human participants are capable of deriving correct predictions for interventions on the basis of observational data (*16*).

In experiment 1, 32 rats were trained on the causal model shown in Fig. 1A, using an observational Pavlovian procedure (*17*). Training consisted of three types of trials interspersed within each session. The first type of trial was presentations of stimulus L (a 10-s flashing light or click train) forward-paired with stimulus T (a 10-s tone or noise); the second was presentations of stimulus L forward-paired with stimulus F (a 10-s delivery of sucrose solution); the third was simultaneous presentations of stimulus N (a 10-s noise or tone) and 10 s of F. We trained each causal link in the common-cause model separately to make it more likely that subjects did not induce a direct link between effects T and F.

Why did the rats not induce that the alternative effect is always absent when the cause and one effect are present (that is, conditioned inhibition)? With few learning trials, rats tend to integrate individual learning relations into a coherent integrated model. Only after many trials do rats encode the explicit absence of the nonpresented cues (*18*). Supporting these findings, the results of all our experiments show that rats induced second-order excitatory rather than inhibitory relations (*19*). Apparently, in the initial phases of learning, rats tend to conservatively treat the absent but expected events as possibly present but missed. A similar ability to combine individually learned causal links into complex causal models has been demonstrated in humans (*20*).

Do rats treat L as a common cause of both T and F, and do they correctly differentiate between seeing and doing with respect to T and N? Rats were allocated to one of four test conditions and were placed in the conditioning chamber with a lever present. This lever had not been present in the observational learning phase, so that no prior instrumental knowledge was available. Rats in condition intervene-T received a 10-s presentation of T each time they pressed the lever. Rats in condition observe-T merely observed presentations of T independently of any emitted lever presses. Conditions intervene-N and observe-N were conducted in an identical fashion, except that N was either the product of an intervention by lever pressing or was observed. We recorded the number of nose pokes into the magazine where F had been delivered during the training phase, to assess the rats' expectation of F.

Causal Bayes nets predict that observing T in condition observe-T should lead the rats to reason that the temporally prior cause L was probably present (but missed), and to consequently expect that F should also be present; therefore, they should emit many nose pokes. In contrast, rats in condition intervene-T should attribute T to their intervention and therefore expect L and consequently its effect, F, to occur with the probability corresponding to the base rate of its cause L. Consequently, we should observe a lower rate of nose poking in condition intervene-T than in condition observe-T. There should not be any difference in rates of nose poking, however, between conditions intervene-N and observe-N. The direct causal relationship should lead the rats to expect F regardless of whether N was observed or intervened on at test. Unlike causal Bayes nets, associationist theories predict equivalent nose poking in the presence of T in both the observe and intervene conditions.

Figure 1B shows the mean rate of nose poking per 10-s presentation of stimuli T and N as a function of test condition (with a maximum rate of 100 nose pokes per presentation). As predicted by causal Bayes nets, rats that produced T through a lever-press intervention (condition intervene-T) made fewer nose pokes than rats that merely observed T (condition observe-T). However, rats that intervened in N (condition Intervene-N), which was trained as a direct predictor of F, did not nose poke less than rats that merely observed N (condition observe-N). [An analysis of the lever press data ruled out selective interference between lever pressing and nose poking (*17*).]

In experiment 1, we observed a dissociation between seeing and doing within the common-cause model, whereas both tasks led to identical expectations with the direct causal link, which is consistent with causal Bayes nets. A critic might point out that we found a dissociation within a complex causal model with two separately learned links (the common-cause model), whereas we found similar responses to the less complex direct link. To rule out complexity or second-order learning as the basis of our dissociation, we compared a common-cause condition with an equally complex causal chain in which the individual causal links were also presented separately (that is, second-order conditioning) (Fig. 2). Whereas causal Bayes nets predict a dissociation between seeing and doing in the common-cause model, no such dissociation is expected for the causal chain. Regardless of whether the initial cause (T) of the chain is observed or generated by means of an intervention, the intermediate (L) and final effect (F) should equally be expected.

In experiment 2a, rats received either common-cause training, as in experiment 1, or causal-chain training, which was identical except that T preceded L during observational learning (*17*). In the test phase, groups

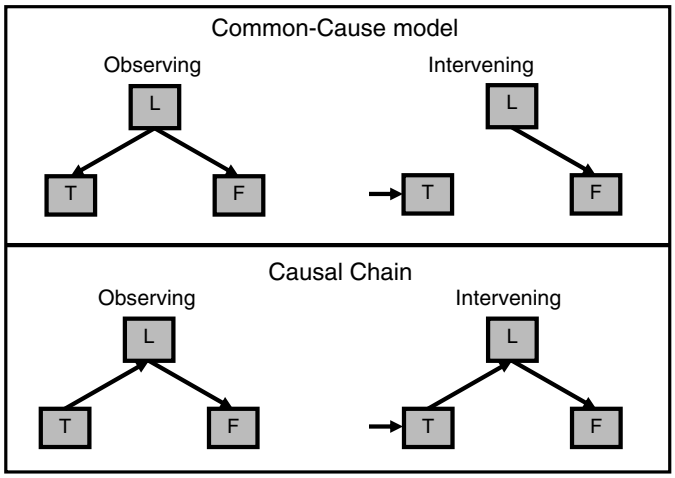

Fig. 2. Common-cause and causal chain models from experiment 2. (**Left**) Observed causal relations. (**Right**) Model modified under the assumption of an intervention in T.

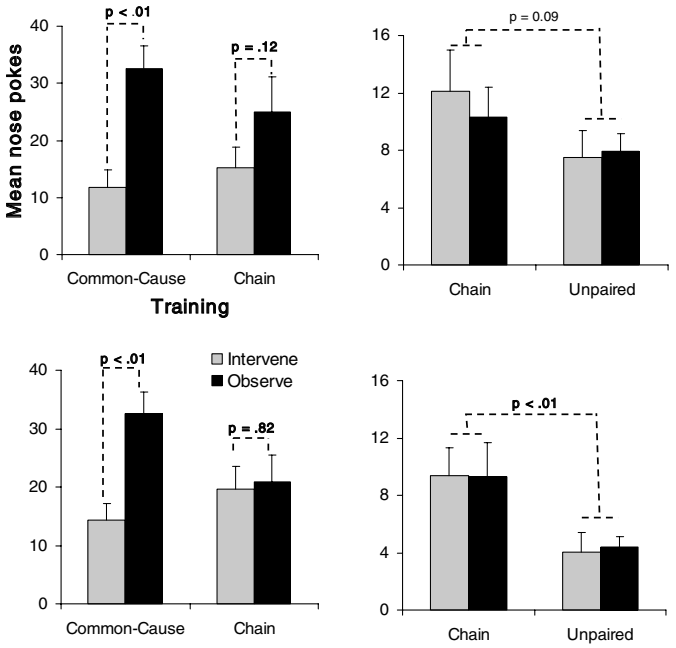

Fig. 3. Experiment 2a (left panels): Mean nose pokes during test stimulus T (top panel) or 10 s after the termination of T (bottom panel) after a lever press (intervene; $P = 0.01$ in both panels) or no lever press (observe; $P = 0.12$ and 0.82 in top and bottom panels, respectively). Common-cause and chain indicate the type of causal model training. Bars indicate SEM. Planned comparisons from two-way ANOVAs are shown. Experiment 2b (right panels): Mean nose pokes during test stimulus T (top panel) or 10 s after the termination of T (bottom panel) after a lever press (intervene) or no lever press (observe). Chain and unpaired indicate the type of causal model training. Bars indicate SEM. $P = 0.09$ and 0.01 in top and bottom panels, respectively, for the main effect of training.

common-cause–intervene and chain-intervene received presentations of T each time the lever was pressed. Groups common-cause–observe and chain-observe merely observed T. We report the number of nose pokes during the 10-s presentation of T and during the 10-s period beginning 10 s after the termination of T (post-T interval 2) for all subjects. In the chain condition, F should rationally be expected between 10 and 20 s after delivery of T (*19*). In contrast, the expected time of delivery of F for rats that received common-cause training is during T itself.

Figure 3 shows the mean rate of nose poking on test trials with T. Group common-cause–intervene nose poked less than group common-cause–observe, which replicates the pattern of experiment 1. In contrast, no difference was found between groups chain-intervene and chain-observe, as predicted by causal Bayes nets.

Rats in group chain-intervene did not nose poke more than did rats in group common-cause–intervene. This low level of responding does not reflect a failure to learn a causal chain, however. Experiment 2b replicated the chain condition and added groups for which T and L were unpaired during observational learning (*17*). Figure 3 reveals no difference between seeing and doing, as predicted by causal Bayes nets. Moreover, responding in the causal-chain groups was higher than in the unpaired groups, which signifies that the rats had indeed learned the second-order chain relations.

A number of researchers have recently concluded that causal reasoning is a faculty that divides humans from animals (*7*, *9*–*11*). The present results cast doubt on that conclusion. With tasks that did not require complex physical knowledge, the experiments have shown that rats grasp the relationship between seeing and doing. Rats made correct inferences for instrumental actions on the basis of purely observational learning, and they correctly differentiated between common-cause models, causal chains, and direct causal links. These results contradict the view that causal learning in rats is solely driven by associative learning mechanisms, but they are consistent with causal Bayes net theories. The core competency of reasoning with causal models seems to be already in place in animals, even when elaborate physical knowledge may not yet be available.

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International Careers Report: Switzerland

Commendable Collaboration

As they seek to compete effectively with other global powers in life science, Switzerland's government and research institutions focus increasingly on encouraging large-scale collaborative projects. BY PETER GWYNNE

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Switzerland has a long history of success in the life sciences. The Alpine nation is the base for several multinational pharmaceutical firms and boasts a network of first rate universities and technical schools. But the fact that Switzerland is small in terms of both geography and population has begun to raise doubts that it can continue to maintain the highest quality of life science research against strong competition from larger nations. The Swiss federal government, along with industrial and academic leaders, has moved to quell those doubts by encouraging extension of a characteristic already ingrained in the Swiss scientific character: collaboration. Government departments, academic centers, industrial research organizations, and private research institutions have started to pool their resources in collaborative research projects designed to ensure that high quality life science research – along with top-notch life scientists – remains in Switzerland.

The collaborative initiatives will plainly take time to produce dividends. But observers are optimistic that they will succeed. "Switzerland still lags behind in the collaboration between universities and industry, with a historical and reciprocal defiance," says Pierre Spierer, dean of the faculty of science and professor in the department of zoology and CONTINUED »

International Careers Report: Switzerland

animal biology at the University of Geneva. "But the focus of research of both academia and industry is becoming closer with the development of genetics in a broad sense, and the situation is improving rapidly." Lutz-Peter Berg, a science attaché in the Swiss Embassy in London, agrees. "Like everywhere in Europe, the attitudes within universities are changing, and the recent success for Swiss startup companies tells me that the environment is good for academic-industrial relationships," he says.


MICHEL AGUET

Sufficient Size

Hanns Möhler, former director of the Institute of Pharmacology and Toxicology at the University of Zurich and Zurich's Federal Institute of Technology (ETH), outlines the problem. "One of the critical points one would have to look at in the long run is whether life science projects in Switzerland are sufficient in size and scope to promote industry in Switzerland," he explains. As a specific example of the concern he notes that Novartis, the giant pharmaceutical firm based in Basel, recently shifted its focus of R&D to Cambridge, Massachusetts. "Does that mean that Novartis is changing its emphasis away from Switzerland, even though it is also expanding in Basel?" Möhler asks. "The move contributed to an ongoing appraisal of the research landscape with the aim of promoting industrial investment in Switzerland."

In part, Novartis's move stemmed from fear that the underlying basis of Swiss life science was in decline. "We in industry, and also people in academia, have a sense that the infrastructure has kind of lagged," says Paul Herrling, head of corporate research. "At a time when the United States National Institutes of Health trebled its budget, the Swiss National Science Foundation's budget has stagnated. We're trying to persuade the government to arrest the stagnation that has gone on too long."

The government has already made moves to arrest the decline. Starting in 2001, for example, it set up a series of what it calls National Centers of Competence in Research (NCCRs). "The whole philosophy is to put together competence that would otherwise be kept isolated," says Pierre Magistretti, co-director of the Swiss Federal Institute of Technology's (EPFL's) Brain Mind Institute. Susan Gasser, director of the Friedrich Miescher Institute for Biomedical Research (FMI), outlines the goals of the NCCRs. "They don't replace the core funding or the core support from the institutes that they involve, but they provide that extra appeal to attract new people and to allow a few individuals to do something beyond what the standard funding would allow," she says.

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LUTZ-PETER BERG

A Recipe Book

Möhler's institute in Zurich illustrates the scope of research supported by the program. "We are involved in the NCCR neuroscience," he says. "We have come up with a whole recipe book of validated targets for drug development that industry can choose from. Several companies have picked it up and are developing agents along those lines. In addition, the NCCR neuroscience projects of Martin Schwab of the Brain Research Institute and Roger Nitsch of the University of Zurich's Division of Psychiatry Research have entered clinical development for the treatment of spinal cord injury and Alzheimer's disease, respectively."

Initial impressions suggest that the centers have started successfully. "They have been very effective at stimulating the life sciences by funding top academic scientists and allowing them to develop collaborative projects," Spierer says. "They have provided an enormous stimulus in a few selected areas," adds Isabel Roditi, director of the University of Berne's Institute of Cell Biology. "They will have long-term impact, as those funded so far have had their second four-year funding approved. Universities are establishing chairs as an indirect result of the process; this will have an impact 20 years from now."

The concept also promises to change the basic approach to research. "The very good thing about the initiative is that it has added a dimension to the individual principal investigator–based research concept, particularly with collaborations across areas of competence," says Michel Aguet, director of the Swiss Institute for Experimental Cancer Research. "The centers not only go across disciplines; they also cover larger areas in the same discipline, going from the laboratory to medical usage, for example."

The NCCRs haven't gained universal approval. "My feeling is that it is a very good initiative in principle," says Hugh MacDonald, associate director of the Lausanne division of the Ludwig Institute for Cancer Research. "But the first round of the NCCRs in biology has tended largely to strengthen preexisting expertise in different institutes, whereas the idea was to develop entirely new areas of research. But it's an important initiative and a lot of money by Swiss standards has been put into it."


PETER VAN BLADEREN

Systems X and Others

Another stimulus for collaborative research focuses on a form of life science that, by its very nature, involves collaboration. "The Systems X initiative plans a national network on systems biology," Spierer says. "Its important financing by the pharma industry is the stamp of future success." The initiative builds on research already under way at the Zurich ETH and the universities of Basel and Zurich. The network could extend in the future to the Lausanne Federal Institute of Technology and other major universities. "It is expected to provide an additional boost and increase the strength of the scientific network CONTINUED »

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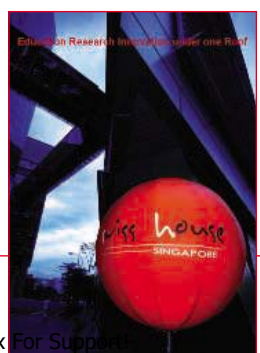

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between more loosely active groups at different places," says Klaus Müller, head of science and technology relations at F. Hoffmann-La Roche (Roche).

A different federal organization, the Commission for Technology and Innovation (CTI), sets out to promote R&D projects that involve public-private partnerships. "Its role is to assure an efficient and result-oriented transfer of knowledge between universities and industries; its objective is to bring science to market," Nestlé's Peter van Bladeren explains.

Other large-scale forms of collaboration have begun on a regional basis. These include the Zurich MedNet, which brings together academic research units and businesses in the greater Zurich area; BioAlps, which combines organizations along the lake shore between Geneva and Lausanne; and BioValley, a multinational collaborative that involves universities and companies in northwest Switzerland, southwest Germany, and eastern France.

Another network involves a field that by its nature involves collaboration among disciplines. The Swiss Institute of Bioinformatics, a network of institutions in Basel, Geneva, Lausanne, and Zurich, hosts several databases, including the SWISS-PROT protein database.


PAUL HERRLING

Industrial Experience

Individual firms and universities have set up their own collaborative research enterprises. An initiative in systems biology that pharmaceutical firm Novartis started with the Universities of Basel and Zurich and the Zurich ETH illustrates the value of the collaborative approach. "The goal was to increase the predictability of our drug discovery," Herrling explains. "Universities were highly interested in this project, as it's the next step for them to understanding life. Both academic and industrial researchers were very keen on getting started on systems biology pathways. This combination of academic and industrial laboratories helps to keep Switzerland at the leading edge of science in competing with the rest of the world."

The Nestlé Research Center provides other examples of academic-industry cooperation in life science research. "We collaborate actively with several universities and Swiss federal institutes of technology," van Bladeren says. "One interesting example is our long-term collaboration with EPFL for the creation and co-financing of a research group in sensory neurosciences. This group is focused on the mechanisms that allow the brain to integrate the various sensorial signals, one of the major challenges that neurobiology faces."

Roche has its own example. "Collaborations with Swiss academia have always been important to us," Müller says. "We have recently set up a large-scale collaboration in systems biology that focuses on pancreatic beta cells, suspected of playing a crucial role in the different stages of diabetes, with the University of Zurich and the Zurich ETH. We have collaborations also with the IBM Research Institute on novel miniaturized assays based on IBM's surface micro/nano-structuring competence. And together with Novartis and the Max Planck Society, we operate a special beamline at the Paul Scherer Institute for structural biology."


PIERRE MAGISTRETTI

From Academia to Private Institutions

Academic institutions have also started to develop their own large-scale collaborative projects. "An important move at the national level is the collaborative program of the University of Geneva, the University of Lausanne, and the Lausanne ETH," Geneva's Spierer says. "As a result, the schools of pharmacy of Geneva and Lausanne were fused and located in Geneva, and Lausanne Federal Institute has absorbed the divisions of chemistry, physics, mathematics, and informatics from the University of Lausanne, and an Institute of Genomics has been created in Lausanne by the three institutions. Achieved last fall, this was the first large restructuring of the landscape of higher education in Switzerland. It is the prototype of future moves, in particular in the Zurich-Basel region."

Similarly, private research institutions emphasize collaborative projects. "Probably 50 percent of our research is collaborative – maybe 25 percent with Novartis and 25 percent with academia," FMI's Gasser says. "We are very open to exchange."

As those examples show, collaboration is hardly foreign to Swiss academic and industrial institutions. "Most definitely there is a culture of collaboration," Gasser points out. "Our institute, funded by the Novartis Research Foundation, has funding opportunities specifically earmarked for industry-academic collaborations. We have about 100 graduate students who go back and forth between academia and industry-funded research institutes. If the cross-feeding starts early with the students, you know it will continue." What is new is the focus on larger-scale collaborations that span several fields, move well along the chain between bench and bedside, and aim for commercial results. "There is already a long tradition of collaboration between universities, research institutes, and industry – for example, in the pharma sector or in engineering," the Swiss Embassy's Berg says. "But of course, Swiss research institutions are currently putting a lot of effort into optimizing technology transfer."


HANNS MÖHLER

No Firm Can Do It All

From the pharmaceutical industry's point of view, the increased focus on collaboration marks a recognition that no firm, however large, can undertake all the research it needs to create new drugs. "The basic research we are not doing in-house has to happen in academic labs," explains Novartis's Herrling. "The only way to be on top of that and to learn is to collaborate with the academics. You need an interactive team of your own scientists working with them; if you just give them money and come back in five years, you won't CONTINUED »

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understand what's happening. So interacting with academia is very much a fundamental aspect of our strategy."

Cooperative research doesn't stop at the border. "I have had two long collaborations with the Swiss Tropical Institute in Basel and the Institute of Biochemistry and Molecular Medicine in Berne," says Roditi of the Institute of Cell Biology. "I have also collaborated with institutes in the United Kingdom, Belgium, and the United States." FMI integrates from the inside. "Our institute has a very international character. We have very few graduate students from Switzerland; most are foreigners," Gasser says. "This exchange of people, ideas, and material is true of Switzerland in general; it's a very open research community. For Switzerland to survive, it has to collaborate. That opened a tradition."

The developing tradition of extra support for collaborative research strikes a particular chord in translational research, which involves cooperation between scientists in the laboratory and the clinic. "Our branch in Lausanne is totally dedicated to immunology, from very basic research to how the immune system develops through to how it functions," the Ludwig Institute's MacDonald explains. "We go from basic research to patients – a nice mixture to have in one organization. We can legitimately say that our research covers the area from bench to bedside, which is unusual for a small institute with fewer than 40 people."

KLAUS MÜLLER

The Emergence of Translational Medicine

Aguet of the Swiss Institute for Experimental Cancer Research takes a similar view. "Translational medicine is getting very important," he says. "We have a good reputation in basic research on cell cycles and cell division. Now we have a major effort in genomic stability, telomerase, and development pathways in cancer. Based on these themes, we wanted to move to medically oriented research. We have moved to cooperate with the local hospital in investigating tumors and cell migration – major aspects of tumor biology that need tumor pathology."

Like other institutions, Aguet's institute seeks scientists prepared to work on collaborative projects. "We will be in partnership with the Federal Institutes of Technology in Zurich and Lausanne, so we are hiring more aggressively," Aguet says. "We are looking for junior and senior people starting now in cancer research. We need to have both very strong basic researchers and researchers with a genuine interest in getting close to the clinic. But it is more difficult to find competent people in the transition field."

Elsewhere, EPFL's Brain Mind Institute has openings in cellular neurobiology and neuropharmacology. "And we have strong development in computational modeling for neuroscience, as illustrated by the Blue Brain supercomputer project," Magistretti says. Roditi's team at the University of Berne plans soon to recruit Ph.D.s and postdocs with training in cell biology, molecular biology, and biochemistry. In industry, says van Bladeren, "Nestlé is moving from being a 'respected and trustworthy food company' to being a 'respected and trustworthy food, nutrition, health, and wellness company.'" That means recruitment of "young and more senior scientists for all our departments in all major areas, from behavioral sciences to life sciences, and from quality and safety to food technology," van Bladeren continues.

ISABEL RODITI

A Feel for Collaboration

Collaborative research obviously demands scientists with a bent toward cooperation. "Collegiality and communication skills are important, along with great curiosity in research," Roditi points out. "In addition, you should not have fixed opinions in advance and you should retain the ability to be surprised." Gasser agrees that "communication skills in any way, shape, or form are essential for our recruits. And above all I look for creativity; I want the bright ideas."

Heiko Bruhn, deputy head of human resources in Roche's Basel office, outlines the typical qualities that Swiss industry seeks in its scientific recruits. "The training should be in depth in a given discipline, such as chemistry, biology, pharmacology, or medicine, but broad and cross-disciplinary so that candidates are fit for the challenging multidisciplinary nature of our modern pharmaceutical research," he says. "Optimally, training should be performed within the contexts of frontier research projects. In the future we will look for people with M.D. and Ph.D. degrees. This 'double education' will help researchers to find the right answers in medicine as well as to understand the methodology of science."

Nationality is relatively unimportant to Swiss recruiters. "We recruit a number of junior scientists, mostly Swiss who have gone abroad for postdocs and have done well," the Ludwig Institute's MacDonald says. "We also have a lot of German and French scientists. The big attraction is limited responsibility outside research. They have virtually no teaching responsibilities; that especially attracts young people." And English speakers who worry about the need to communicate in other languages need fear no more. FMI provides a typical example. "In Switzerland the scientific language is English," Gasser says. "The whole institute speaks English. I've hardly spoken German since I came here from the University of Geneva a year ago."

PIERRE SPIERER

Spreading the Word

The Swiss government, meanwhile, continues to promote its support of collaborative research. "It is stepping up its efforts to keep Swiss science globally connected by setting up a network of international science and technology offices and dedicated 'science consulates' around the world," says Berg of the London Embassy. Their objective: "To facilitate international collaboration and mobility."

A former science editor of Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

Director

ICSU Regional Office for Asia and the Pacific

The International Council for Science (ICSU) invites applications from citizens in developing countries of Asia and the Pacific for the post of Director of its Regional Office for Asia and the Pacific, which is being established at the Academy of Sciences Malaysia, Kuala Lumpur, Malaysia.

ICSU is a non-governmental organization consisting of 104 National Members and 29 Disciplinary Scientific Unions. Further information about ICSU can be found at **www.icsu.org**

This Regional Office will be responsible for the promotion of increased participation of the region in ICSU programmes and activities. Thus, the Office will assist in strengthening science and scientific capacity building in Asia and the Pacific.

The Director will be responsible for the activities of the Regional Office under the direction of the ICSU Executive Director. The initial staff of the Office will consist of a total of three persons. The Director will be appointed for a term of three-years, renewable, under the general conditions applicable to staff at Academy of Sciences Malaysia.

The successful candidate will have an MSc/PhD degree in a relevant discipline and be fluent in written and spoken English. The candidate should have experience of international research collaboration and writing of grant proposals. The monthly salary range will be RM 10,000-15,000 with the maximum equivalent to a senior professor in the Malaysian system. The Director will be employed by the Academy of Sciences Malaysia but the Director and the Regional Office are directly responsible to the Executive Director of ICSU. Strategic and work plans are decided on by the ICSU Regional Committee for Asia and the Pacific.

Application including (i) CV, (ii) a brief note outlining the strengths the candidate can bring to the ICSU Regional Office and (iii) names and addresses of three referees should be sent to: Professor Thomas Rosswall, Executive Director, International Council for Science (ICSU), 51 boulevard de Montmorency, FR-75016 Paris, France, e-mail: **thomas.rosswall@icsu.org**, tel. **+33 (0)1 45 25 03 29**, fax. **+33 (0)1 42 88 94 31**.

Applications should be received no later than **15 March 2006.** It is expected that the Director will start his/her appointment as soon as possible and no later than 1 July 2006.

UNIVERSITÉ DE GENÈVE

UNIVERSITY OF GENEVA

The Department of Molecular Biology seeks a

Full , Associate, or Assistant Professor in Molecular Biology
(Professeur ordinaire, adjoint ou assistant)

The successful candidate is expected to maintain or establish a strong research program in molecular life sciences. Candidates studying important biological problems with novel chemical, genetic or biophysical approaches will be given special consideration. Undergraduate teaching in French is required, but a grace period can be granted. The earliest starting date is 1 October, 2006. Candidates holding a Ph.D. or equivalent degree should send their application, including CV, list of publications, and statement of research interests to the Dean of the Faculty of Sciences, 30, Quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland, and as a single PDF file to **Annick.DeMorsier@sciences.unige.ch**

Deadline for receipt of applications is 1 June, 2006. Further information can be obtained from the director of the Department of Molecular Biology (David Shore, email: **David.Shore@molbio.unige.ch**). Applications from women are particularly welcome.

PHILIPPS-UNIVERSITÄT MARBURG

Professor of Chemical Biology

The Faculty of Chemistry has a vacancy for a professor of chemical biology.

Salary scale and conditions: W 3

Starting date: as soon as possible by arrangement

The person appointed will be expected to teach courses to students with chemistry as a main subject and a minor subject. The appointee will also be expected to cooperate in the following research field: functional chemical-biological hybrid compounds.

Special conditions:
The conditions of employment set down in paragraph 71 of the Hessen State Law on Higher Education will apply. Initially the person appointed will receive a three year contract unless the person was previously employed at a university in an academic position for at least six years. Subject to evaluation, the position may become tenured.

Applications with usual documents accompanied by offprints of a maximum of 5 publications should be sent by mail to: **Präsident, Philipps-Universität, Biegenstraße 10, D-35032 Marburg**.

Closing date: March 31, 2006

Philipps-Universität Marburg is an equal opportunities employer.

University of Zurich

The Division of Psychiatry Research of the University of Zurich is looking for:

Graduate Student 100%

for participation in neurobiological research projects in the field of biomarker discovery in CSF and plasma for diagnosis of Alzheimer's Disease.

The successful candidate should be familiar with biochemical methods such as Western Blotting, Chromatography, ELISA, Mass Spectrometry and Proteomic-Technologies.

Clinical Research Fellow 100%

for participation in clinical research projects in the field of molecular in vivo imaging of pathology in patients with Alzheimer's Disease by MRI and PET/CT.

The successful candidate should be a MD and have clinical experience in psychogeriatrics or neurology.

Please send your application documents to:

Estelle Obrist, Executive Assistant,
Division of Psychiatry Research,
Psychiatric University Hospital, University of Zurich,
Lenggstrasse 31, P.O. Box 1931,
CH-8032 Zurich, Switzerland.

FACULTY POSITIONS
DEPARTMENT OF PHYSICS
THE UNIVERSITY OF TEXAS AT AUSTIN

The Department of Physics at The University of Texas at Austin is seeking candidates for tenure track assistant professorship positions in physics starting in September 2006. In special cases, appointments at more senior levels will be considered. Successful candidates will assume full teaching responsibilities for undergraduate and graduate courses in the Department of Physics and are also expected to conduct vigorous research programs. Research areas of current highest priority for the Department are Biophysics/Soft-Condensed Matter Experiment, Cosmology/Relativity/Theoretical Physics, AMO/Quantum Information Science, Condensed Matter/Nanoscience Experiment, and Particle/Astrophysics Experiment. Outstanding candidates in other areas of departmental focus will also be considered. Excellent English language communication skills are required. Applicants must have a Ph.D. (or equivalent) and a demonstrated potential for excellence in teaching and research.

Interested applicants should send a curriculum vitae, a list of publications, a statement of research interests, a research plan, and should arrange for at least five letters of recommendation to be sent to: **Prof. John T. Markert, Chair, Department of Physics, The University of Texas at Austin, 1 University Station C1600, Austin, TX 78712-0264**. Review of completed applications has already begun and is ongoing.

The University of Texas at Austin is an Equal Opportunity/Affirmative Action Employer.

ACADEMIC

ASSISTANT/ASSOCIATE/FULL PROFESSOR (2 POSITIONS)

The department of Biological Sciences and the Border Biomedical Research Center (BBRC) at the University of Texas at El Paso (UTEP) is seeking pathogenic microbiologists/virologists at the junior and senior professor level who are committed to research and teaching.

QUALIFICATIONS: Ph.D. or M.D. in related field and post-doctoral training required. Applicants should possess the ability to develop an independent extramurally funded research program in area related to infectious disease/bioterrorism/vaccine development. Researchers requiring BSL-3 facilities are particularly encouraged to apply. Preference will be given to candidates whose research interests fit with the mission statement of UTEP and the BBRC www.utep.edu/biology/general/info.htm.

APPLICATION: Interested candidates should submit a curriculum vitae, a brief statement of research and teaching interests and contact information for three references to: Siddhartha Das, Ph.D., Chair, Microbiology/Virology Search Committee, Department of Biological Sciences, University of Texas at El Paso, El Paso, TX 79968-0519. Email: sdas@utep.edu. Review of applications will begin immediately and will continue until the positions are filled. Posted January 18, 2006.

OUR TIME UTEP IS NOW

The University of Texas at El Paso is an Equal Opportunity/Affirmative Action Employer.

www.utep.edu

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Research Leadership 'Next Phase' Appointments

Griffith University has created Strategic Research Programs in its areas of key research strength.

A number of 'Next Phase' appointments will be made to ensure that Griffith University continues to grow as a research intensive University of world class standing.

The University seeks researchers of international standing, or who have a demonstrated capacity to achieve that status to join these dynamic interdisciplinary research teams.

Professor or Associate Professor Water Sciences

Faculty of Environmental Sciences

2 appointments - Nathan campus and Gold Coast, Queensland, Australia

The Strategic Research Program in Water Sciences builds on Griffith's long-established record of research excellence in the environmental sciences. The particular strengths include: freshwater and coastal ecosystems, and catchment and water resource management. Water research at Griffith University is currently focused in two Centres - Riverine Landscapes led by Professor Stuart Bunn (Nathan) and Aquatic Processes and Pollution led by Professor Joe Lee (Gold Coast).

The University is also a partner in a new International Water Centre, the eWater Cooperative Research Centre, and is currently leading a bid to establish a major water research facility to be based at the Gold Coast campus.

The two researchers are expected to take lead roles in one or more of the following areas - cultural and ethical aspects of water; economics, including valuation of ecosystem goods and services; aquatic biodiversity and conservation; aquatic ecosystem health assessment, including ecotoxicology; innovative water quality monitoring; water-borne pathogens and diseases.

Successful candidates will have:

- international standing in their field or demonstrated potential to achieve international status
- a history of excellent research and publications
- a successful record for winning competitive grants
- an ability to work cooperatively with other members of their Strategic Research Program and related centres
- innovative and active research plans in the areas to which the Strategic Research Program is committed.

The appointments are on a continuing basis and appointees will be research and research leadership focused for at least the first five years.

Salary range: Professor A$119,193 per annum
Associate Professor A$92,531 - A$101,941 per annum.

Plus 17% employer superannuation contribution

For a confidential discussion and further Information please contact the Consultant assisting the University:
BSP EXECUTIVE SEARCH
Professors of Water Science
Dr Jim Sait + 61 2 8904 1532 and + 61 400 292 322 or jim@bspes.com
Closing Date: 17 March 2006

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1 | Go to **www.griffith.edu.au/hrm/employment/** for further information on the position and selection criteria.
2 | Follow the specific application process for that position.
3 | Applications can be lodged in electronic or hard copy form. All applications will be acknowledged.

TMP D003398

GOLD COAST LOGAN MT GRAVATT NATHAN SOUTH BANK | **www.griffith.edu.au**

NIDDK

Staff Scientist Position
Isotope Ratio Mass Spectrometry Laboratory

The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK) is establishing a new core facility named the Isotope Ratio Mass Spectrometry Laboratory and invites applications for a mass spectrometrist. The candidate will have the opportunity to set up the mass spectrometry laboratory and then operate and maintain the instrumentation, design and execute experiments in close cooperation with clinical investigators, and participate in the interpretation of the results. The candidate will participate in research meetings and communicate laboratory activities and findings orally and in writing as required. The candidate will work on cutting-edge research in a network of internationally renowned scientists and will enjoy the benefits of joining a supportive scientific community.

Experience in gas chromatography, liquid chromatography and isotope ratio mass spectrometry techniques is required. Prior knowledge of the theoretical and practical matters related to preparation and analysis of biological samples for isotopically labeled glucose, lipid and doubly labeled water experiments is preferred.

This core facility is located on the main NIH campus in Bethesda, Maryland, a suburb of Washington, D.C. Clinical investigators comprising the major points of contact for this position perform patient-oriented research in a number of areas of endocrinology and metabolism with a special emphasis on studies of obesity. Salary and benefits will be commensurate with the experience of the applicant.

The successful candidate will have a Masters or Ph.D. in analytical chemistry, biochemistry or a related field, with knowledge and interest in the following: liquid/gas chromatography and isotope ratio mass spectrometry. Interested candidates should submit a curriculum vitae, bibliography, a summary of accomplishments and arrange for three letters of reference to be sent to:

Ms. Jackie Collier, Office of the Director, Division of Intramural Research, NIDDK, National Institutes of Health, Bldg. 10, Room 9N222, MSC 1818, 9000 Rockville Pike, Bethesda, MD 20892.

NIDDK

Immunologist Tenure Track Position

The Diabetes Branch of the National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health, is recruiting a qualified and experienced M.D. or Ph.D. with an interest in pursuing immunological research designed to either elucidate the autoimmune process underlying Type 1 diabetes mellitus (T1DM), develop assays for monitoring that autoimmune process, and/or test immunological interventions to ameliorate the process. The tenure track incumbent would manage his or her own research program but would also be expected to collaborate with existing investigators pursuing basic and clinical research, both within the Diabetes Branch and the broader NIDDK/NIH community.

The Diabetes Branch conducts basic and clinical research designed to better understand the immune-mediated beta cell destruction that underlies T1DM, and/or the target of this autoimmune process - the pancreatic beta cell. Toward that end, Branch investigators have pursued studies ranging from basic biochemical laboratory projects to studies involving a mouse model developed within the Branch, to non-human primate studies, and to clinical and epidemiological studies. While the path toward a T1DM cure remains blocked by several hurdles, the pre-eminent one is an incomplete understanding of the chronic autoimmune process that causes the disease. In addition, investigators interested in overcoming that autoimmune illness are plagued by the lack of assays to follow the autoimmune response and safe therapies for interdicting the autoimmune process.

The Diabetes Branch of NIDDK is located on the main campus of the NIH in Bethesda, Maryland, a suburb of Washington, D.C.

Competititve salary and benefit packages are available. Interested applicants should send a Curriculum Vitae and list of publications, copies of three major publications, a summary of research accomplishments, a plan for future research, and three letters of recommendation by **April 15, 2006** to **Dr. David Harlan, Chair, Search Committee, c/o Ms. Guerdy Toussaint, Diabetes Branch, NIDDK, NIH, Building 10 CRC, Room 5W-5940, 10 Center Drive, Bethesda, MD 20892-1453**.

WWW.NIH.GOV

NIDDK POSTDOCTORAL FELLOWSHIPS IN SIGNALING PATHWAYS AND DISEASE

Postdoctoral Fellowships are available in the Division of Intramural Research, NIDDK, NIH. The intramural program of the NIH offers an outstanding research environment and has been rated by *The Scientist* as one of the top places for post-doctoral fellows to work. It is located on the main campus of the NIH in Bethesda, Maryland, a 20-minute ride from Washington, D.C. Applications are invited from individuals of the highest caliber who have obtained a Ph.D., or M.D. degree within the last 5 years. Salary and benefits will be commensurate with the experience of the applicant. Interested candidates should send their curriculum vitae, list of publications, a letter stating their interests and the names of three references to the appropriate individual by e-mail or by regular mail. Positions are available in the following areas:

GNAS imprinting and role of the G protein $G_s\alpha$ in metabolic regulation

GNAS is a complex imprinted gene with multiple gene products including the stimulatory G protein $G_s\alpha$ which is the underlying disease gene for several disorders of hormone signaling. Our laboratory studies the mechanisms of *GNAS* and $G_s\alpha$ imprinting in mouse models and in patients with *GNAS* imprinting defects. We are also studying the role of $G_s\alpha$ and other *GNAS* gene products in metabolic regulation using several germline and tissue-specific *Gnas* knockout mouse models. **(Lee S. Weinstein, MD, Bldg. 10 Rm. 8C101, NIDDK/NIH, Bethesda, MD 20892; leew@amb.niddk.nih.gov).**

Molecular Genetics of Signal transduction/tumorigenesis

We have helped discover two genes causing hereditary endocrine cancers (*MEN1* and *HRPT2*). Our efforts are directed at the function of menin, the encoded protein from the *MEN1* tumor suppressor gene. With protein-protein interaction methods, we have identified four menin partners. Our lab also collaborates extensively with other labs in the NIDDK, NCI and NHGRI to study the function of menin in Drosophila and in knockout mice. This position involves creativity and lab expertise with cutting-edge methods. (**Stephen Marx MD, Bldg. 10 Rm. 9C101, NIDDK/NIH, Bethesda, MD 20892; StephenM@intra.niddk.nih.gov**).

Molecular Biology AND Genetics

A post-doctoral position is available to study the mechanism of BRCA1-associated tumorigenesis **(visit http://www.niddk.nih.gov/intram/people/cdeng.htm for details). A strong** background in molecular biology and/or signal transduction is required. **(Chuxia Deng, PhD, Building 10, Room 9N105, 10 Center Drive, Bethesda, MD 20892-1812; ChuxiaD@bdg10.niddk.nih.gov; Tel: (301) 402-7225; Fax: (301) 480-1135).**

The National Institute of Allergy and Infectious Diseases (NIAID), a major research component of the NIH and the Department of Health and Human Services, is recruiting for a Post-doctoral Fellow or Research Fellow. The position is available in the Bacterial Toxins and Therapeutics Section of the newly formed Laboratory of Bacterial Diseases (LBD). The LBD will be located in new and well-equipped facilities on the main NIH campus. Scientists with a M.D., Ph.D., or DVM are eligible. The Research activity involves (1) characterization of bacterial virulence factors, particularly toxins, proteases, and hemolysins, in both cultured cells and animal infection models; (2) structure-function analyses of protein toxins; (3) study of gene regulation in *Bacillus anthracis*; (4) identification of new candidate vaccines and therapeutics for anthrax; and (5) development of toxin variants that target specific cell types, e.g., malignant cells. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to development of vaccines and therapeutics, and it provides excellent training for newly graduated Ph.D. scientists, for postdoctoral scientists, and for MD's at all levels of training who plan a career in research in infectious diseases. The salary range for Post-doctoral Fellows is $38,500-56,900, depending on experience. Research Fellow applicants should have three or more years of post-doctoral experience; the salary range is $40,974-72,990. Applicants with an MD degree are eligible for the NIH Loan Repayment Program. Applicants should send their curriculum vitae, a letter of interest, and names and addresses of three (3) references to **Stephen Leppla, 30 Convent Drive, MSC 4349, Building 30, Room 303, Bethesda, MD 20892-4349, FAX: (301) 480-0326, email: sleppla@niaid.nih.gov**

Postdoctoral Fellowships
Intramural Research Program
National Institute of Mental Health
Bethesda, Maryland, USA

NIMH National Institute of Mental Health

The National Institute of Mental Health (NIMH), Section on Neural Gene Expression, seeks two broadly trained neuroscientists with backgrounds in rodent behavioral testing. Experience with biochemistry and/or molecular biology is highly desirable. The fellows will study knockout and transgenic mice as part of investigations into the regulation and roles of vasopressin, oxytocin and their receptors in the brain.

Positions are available spring or summer 2006 and 2007.

Applicants should have a Ph.D. or M.D. degree with less than five years of postdoctoral experience.

NIMH is a major research component of the National Institutes of Health and the Department of Health and Human Services, which have nationwide responsibility for improving the health and well being of all Americans. Interested applicants should send curriculum vitae, bibliography, together with three letters of reference to: **Dr. Scott Young, Chief, Section on Neural Gene Expression, NIMH, 9000 Rockville Pike, Building 49, Room 5A-56, Bethesda, MD 20892-4483, USA; or e-mail to wsy@mail.nih.gov**

JOHN B. PIERCE LABORATORY/ YALE SCHOOL OF MEDICINE/ YALE UNIVERSITY

The John B. Pierce Laboratory, an endowed research institute affiliated with Yale University, seeks to expand its programs in body energy balance and sensory neuroscience by adding scientists with research programs in environmental physiology and in chemosensation or somatosensation.

ENVIRONMENTAL PHYSIOLOGY

To complement and extend its research program in body energy balance, the Laboratory seeks to add up to three investigators working in research areas of programmatic interest, such as human and/or animal thermoregulatory, cardiovascular, and metabolic responses to environmental stimuli. Joint appointments are anticipated in the Department of Epidemiology and Public Health, Yale University School of Medicine.

CHEMOSENSORY/SOMATOSENSORY NEUROSCIENCE

To complement and extend its research program in chemosensory and somatosensory neuroscience, the Laboratory seeks to add up to three investigators working in research areas of programmatic interest, such as functional neuroimaging of brain processes, peripheral/central coding mechanisms, and central neural processing in relation to energy demand (e.g., food intake, thermoregulation). Joint appointments are anticipated in the Department of Neurobiology, Yale University School of Medicine.

All candidates should have strong evidence of the ability to obtain external funding. Ranks of the appointments are open.

The Laboratory offers competitive salary, benefits, and start-up, as well as an outstanding work environment. Applicants should submit a CV, description of research interests, set of representative publications, and names of at least three references to: **Chair, Environmental Physiology Search, or Chair, Sensory Neuroscience Search, The John B. Pierce Laboratory, Inc., 290 Congress Avenue, New Haven, CT 06519.** Review of applications will begin **February 15, 2006**, and continue until the positions are filled.

EOE/AA **www.jbpierce.org**

University of Connecticut Endowed Chair in Mechanistic Toxicology

Department of Pharmaceutical Sciences Pharmacology and Toxicology Discipline

The Department of Pharmaceutical Sciences is pleased to announce the creation of the Boehringer-Ingelheim Endowed Chair in Mechanistic Toxicology to be filled at the level of Professor. We are seeking a scientist with a strong background in the field of drug-induced liver injury with a focus on novel and integrative approaches for the early detection of drug liver toxicity. Other areas of interests include species differences in susceptibility to hepatotoxicity by pharmaceuticals as well as development and validation of new biomarkers to better predict human liver toxicity. The successful candidate's research expertise will complement the interests of the faculty in the Department of Pharmaceutical Sciences, which includes integrative approaches to elucidate mechanisms of drug or toxicant action, drug discovery and design, and pharmaceutical technology. The Department is housed in a new 200,000 square foot state-of-the-art-building in the science quad of the University of Connecticut and encourages faculty interdisciplinary interactions with other programs of the University such as the Molecular and Cell Biology Program, the Center for Regenerative Biology and the Stem Cell Initiative. The candidate must have an established record of leadership in the field of liver toxicology and is expected to head a strong extramurally funded research program and to participate in the Department's graduate and professional programs. Applicants must possess a Ph.D. degree or equivalent.

Review of applications will begin **April 28, 2006** and the search will continue until the position is filled. Applicants should submit electronically a curriculum vitae, brief statement of research and teaching interests, and names and e-mail addresses of three references to **José E. Manautou, Search Committee Chair**, at **jose.manautou@uconn.edu**.

In keeping with our commitment to build a culturally diverse community, the University of Connecticut invites applications from women, people with disabilities, and members of minority groups.

Director of Metabolomics
Roy J. Carver Biotechnology Center
University of Illinois at Urbana-Champaign

Proposed Starting Date: As soon as possible after closing

Qualifications: M.S. in chemistry or life sciences related field is required, Ph.D. is preferred. Demonstrated experience required using analytical instrumentation that includes HPLC, HPLC-MS, and GC-MS. Familiarity with robotic microarray printing, robotic colony picking/re-array, and metabolite production through microbial fermentation is desired. Successful candidate must have excellent communication and managerial skills.

Responsibilities: To direct and oversee the operation and continued development of the Metabolomics Center that supports the detection, isolation, and characterization of metabolites.

Type of Position and Salary: Full-time academic professional position. Salary commensurate with experience.

Application: Send letter of application, academic transcript, three letters of reference, and resume to:

Rhonda Lipking
Biotechnology Center
901 S. Mathews
103 Observatory
Urbana, IL 61801
Phone: (217) 333-1695
Email: lipking@uiuc.edu

Questions regarding this position should be directed to:
Dr. Mark Mikel
Associate Director
Biotechnology Center
Phone: (217) 244-0144
Email: mmikel@uiuc.edu

Deadline: For full consideration, application must be received by **5:00 pm, Friday, March 10, 2006**.

The University of Illinois is an Affirmative Action/ Equal Opportunity Employer.

POSTDOCTORAL POSITIONS

The Defense Biology Division of the Biosciences Directorate at Lawrence Livermore National Laboratory (LLNL) has openings for two Postdoctoral Research Staff members to conduct research in the areas of innate immunity and microbial pathogenesis.

Areas of specialization must include one or a combination of the following areas of research: Immunology (emphasis on initiation of innate immune responses), infectious diseases, microbiology, host-microbe interactions, comparative genomics, microbial physiology. Strong record of publications and scientific achievements is required and expertise in cell culture, animal work, DNA microarrays and imaging techniques (confocal, electron microscopy) is highly desirable.

LLNL offers a challenging environment and a competitive salary/benefits package. To view and apply for this job, go to **http://jobs.llnl.gov,** Search by job #004706. When applying and prompted please mention where you saw this ad. LLNL is operated by the University of California for the Department of Energy. We are proud to be an equal opportunity employer with a commitment to workforce diversity.

University of California
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Science Careers Forum

- How long should it take to get my Ph.D.?
- Academia or industry?
- What will make my resume/cv stand out?
- How do I negotiate a raise?

Connect with Experts

Moderator Dave Jensen
Industry Recruiter

Mr. Jensen has over 20 years of experience in human resource consulting and staffing for the biotechnology and pharmaceuticals industry.

Adviser Bill Lindstaedt
Director,
UCSF Career Center

Mr. Lindstaedt has been providing career related advice to scientists and engineers for nearly 15 years, with a particular emphasis on working with graduate-level trainees in the life sciences.

Adviser Naledi Saul
Assistant Director,
UCSF Career Center

Ms. Saul has 7 years of career counseling with 4 years focused on counseling graduate students and postdocs in the biomedical and health sciences. Her forte is working with scientists pursuing careers in the public health arena.

Adviser Jim Austin
Editor, Science's
Next Wave

Dr. Austin has a Ph.D. in physics and worked in academia before coming on board to write about traditional and nontraditional career paths for scientists.

Visit www.sciencecareers.org and click on Career Forum

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Faculty Position

Antitumor Assessment Core Facility Head

The Molecular Pharmacology and Chemistry Program of Sloan Kettering Institute seeks a manager for its Antitumor Assessment Core Facility. The manager is responsible for a small group of investigators that provides resources and technical expertise in testing new anti-cancer agents in small animals. This is a non-tenure track position. Applicants must have an MD or PhD degree in a relevant field, extensive experience with mouse tumor models and in preclinical experimental therapeutics, a record of productivity, and management experience.

Candidates should send by March 20, 2006 their curriculum vitae and the names of 3 references to: **David Scheinberg, MD, PhD, Chairman, Molecular Pharmacology and Chemistry Program; Memorial Sloan-Kettering Cancer Center; c/o Claudia Little-Box 531; 1275 York Avenue; New York, NY 10021;Email: littlec@mskcc.org.** Memorial Sloan-Kettering Cancer Center is an affirmative action, equal opportunity employer.

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PRE AND POST DOCTORAL FELLOWSHIPS

The University of Texas Health Science Center at San Antonio invites applications for Pre and Post Doctoral fellowships working in research related to Latino mental health. Since more effort is needed to elucidate the mental health issues of the largest minority population in the United States, the National Institute of Mental Health has funded our program.

The program offers a 2-3 year Neuroscience-oriented curriculum and a 1-3 year Clinical-oriented curriculum. Fellows that are accepted into these programs will benefit from already established clinical and genetics databases on Schizophrenia at the UTHSCSA and the Southwest Foundation for Biomedical Research, a well equipped Neurogenetics Laboratory for Biochemistry and Molecular Biology with established banks of DNA samples and cell lines, access to the Research Imaging Center outfitted with state of the art neuroimaging equipment, ongoing research in genetics of Bipolar Disorder, collaborations with several sites in the United States and Latin America and a large Latin population available for research.

Depending on the program of interest, applicants must be graduates of a master's level program in Psychology, Social Work or related field; graduates of an accredited Psychiatry Residency program or from a PhD Psychology program; or graduates of a PhD program in one of the Biomedical Sciences. Salary will be based on qualifications. Interested applicants should request an application via email to Rolando A. Medina, MD, MPH, **medinar@uthscsa.edu**. Upon arrival of your request, we will send you the application and additional information that is required. The deadline is **March 10, 2006**.

The UTHSCSA is an Equal Employment Opportunity/Affirmative Action Employer. All postdoctoral appointments are designated as security sensitive positions.

Our boundaries may surprise you.

Expand your career of discovery

R&D SCIENTISTS (3 OPENINGS)

Promega is seeking creative research scientists to join our growing R&D team developing innovative technologies and products for life science researchers. Ph.D. level scientists should have laboratory experience in molecular biology, biochemistry and mammalian cell biology. They should be able to effectively analyze scientific literature and create innovative approaches to experimental design. Individuals must have effective communication skills and the ability to work independently and in interactive teams.

Senior Scientist Cellular Imaging

Ph.D. level must have postdoctoral experience in mammalian cell biology and a proven track record in carrying out laboratory research using live cell imaging technologies. We are particularly interested in candidates with experience using fluorescent probes to analyze cellular processes and protein function. Experience in working with ion channels is a plus.

Senior Scientist/Group Leader: Cell-Free Protein Expression

Ph.D. scientist with project management experience and a proven track record in laboratory research studying protein translation. We are particularly interested in candidates with experience using cell free protein expression systems. Position also requires experience in managing a group of research scientists.

Senior Scientist/Group Leader: Protein Structures

Ph.D. scientist with project management experience and the technical ability to create novel protein and enzyme structures. Directed evolution and protein structure analysis experience is required. Proven success championing commercial projects or funding research through peer-reviewed grants required. Position also requires experience in managing a group of research scientists.

Interested candidates please forward resume to: Human Resources, 2800 Woods Hollow Road, Madison, WI 53711; Fax: 608-277-2512; email: hr@promega.com Please indicate for which position you are applying.

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ENGINEERING FACULTY

The Pratt School of Engineering at Duke University is seeking applications for a tenure or tenure-track faculty opening intended to impact the growth of Pratt's four Strategic Initiatives in Bioengineering, Materials Science and Engineering, Photonics, and Environmental Engineering. Qualified candidates must have a Ph.D. or the equivalent in Engineering or the physical sciences. While the search is open to all qualified applicants regardless of rank or experience, we are particularly interested in candidates with demonstrated vigorous research and teaching interests that align well with one or more of Pratt's Strategic Initiatives. The primary academic appointment of the successful candidate will be in one of Pratt's four engineering departments: Biomedical Engineering, Civil & Environmental Engineering, Electrical & Computer Engineering, or Mechanical Engineering & Materials Science.

Please send a letter of application, description of research program, and teaching portfolio to: **Chair, Target of Opportunity Search Committee, Department of Biomedical Engineering, Box 90281, Duke University, Durham, NC 27708-0281. Email: elaine.ruger@duke.edu**.

The Pratt School of Engineering and Duke University are Affirmative Action/Equal Opportunity Employers that are committed to increasing the cultural and intellectual diversity of its faculty.

Duke University

PHYSICIST SCIENTIST / ENGINEER

BERKELEY LAB

ADVANCED LIGHT SOURCE DIVISION

Reporting to the Division Deputy for the Scientific Support Group (SSG) of the Advanced Light Source (ALS) Division, the incumbent functions as an experimental physicist, supporting and planning experiments to meet the SSG's programmatic plans and R&D goals. He/she is responsible for the operation, maintenance, and upgrades of ALS beamline 10.0.1 and its atomic and molecular physics endstations. To learn more about the ALS please visit our website at **http://www-als.lbl.gov.**

Duties: The candidate will participate in planning and performing forefront investigations in atomic and molecular scientific research. He/she will provide support for the Scientific Support Group experimental program including operation of undulator beamline 10.0.1 and its endstations, support of the atomic and molecular physics users on BL 10.0.1, scheduling of beamline operations, adherence to environment, safety, and health policies and procedures, ensuring facility and equipment maintenance, and participation in planning and implementation of R&D upgrades. The candidate will collaborate with various physicists, engineers, and technical support staff to perform planned experiments. It will be necessary to disseminate experimental and research results via publications in refereed journals, present results via verbal presentations at various meetings, conferences, and peer reviews. He/she will participate in ALS R&D planning efforts and contribute to selected programmatic projects and collaborations in R&D activities, and must maintain current knowledge of and implement relevant Laboratory, Division, Program Conduct of Operations, and Quality Assurance policies and procedures for their activities.

Qualifications: Experience with synchrotron radiation techniques applied to the study of atomic and molecular physics is essential. Knowledge of atomic and molecular physics and chemistry as well as a thorough understanding of modern instrumentation techniques applied to these areas is required. The candidate must be independent, innovative, and able to design, guide, and carry through an experiment from initiation to meaningful conclusion in collaboration with a team of engineers, physicists, technical support staff, post docs, and students. Proven ability to pursue new areas of research in physics involving sophisticated instrumentation including vacuum, charged particle detection, and momentum determination is essential, as is knowledge of the characteristics and properties of synchrotron radiation sources. Effective verbal and written communication skills are necessary, and the candidate must have a Ph.D. or equivalent in Physics, Chemistry, or Materials Science.

Apply online at: **http://jobs.lbl.gov**, select "Search Jobs", enter **018721** in the keyword search field, select the "Upload Your Resume" option, and follow the on-line instructions to complete the application process. Enter "Science Magazine" as your source. EOE

UCLA

INSTRUCTOR POSITION AVAILABLE
MOLECULAR, CELL, AND DEVELOPMENTAL BIOLOGY

Deadline: March 15, 2006

Position: Full time position. Provide instruction and general education curriculum development in the Department of Molecular, Cell, and Developmental Biology at UCLA (Fall, Winter and Spring Quarters and Summer Sessions). Percentage of employment will vary according to number of courses taught.

Duties: Teach science general education lecture courses in the areas of Stem Cells, Biology of Cancer, and AIDS; plan and coordinate course activity, outside speakers, and service learning components for multiple courses; supervise course reader staff; manage the development and maintenance of course materials; develop computer applications for the classroom; and maintain office hours.

Qualifications: Ph.D. degree in the biological sciences; substantial familiarity with biology concepts related to stem cells, cancer biology, and AIDS. Demonstrated experience in undergraduate teaching at the university level; demonstrated knowledge of pedagogy as related to instruction in biological sciences. Level of appointment and salary commensurate with qualifications, experience and duties.

Application: Please send curriculum vitae, written statement of teaching interests and background, and the names, addresses, and telephone numbers of three references. Applications should be mailed to: **UCLA MCDB Lecturer Search, ATTN: Ms. Grace Angus, 621 Charles E. Young Dr. South, Box 951606, Los Angeles, CA 90095-1606.**

The University of California is an Equal Opportunity Employer committed to excellence through diversity.

U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment (NCEA)

Supv. Biologist/Toxicologist/Health Scientist/Physical Scientist/Mathematical Statistician
Ez hire Announcement #RTP-DE-2006-0048 or RTP-MP-2006-0080

The U.S. Environmental Protection Agency is seeking highly qualified applicants for two Branch Chief positions with the National Center for Environmental Assessment (**http://cfpub.epa.gov/ncea/**) which are located in Cincinnati, Ohio. Duties include supervision and leadership of an interdisciplinary team of scientists conducting high-profile human health and ecological assessments and developing cutting-edge risk assessment methods, with emphasis on water quality and hazardous waste.

Excellent benefits: The selected candidate will be eligible for a full benefits package, including paid relocation, health insurance, life insurance, retirement, and vacation and sick leave. This is a permanent, full time position. U.S. citizenship is required.

Salary Range: The salary range is $91,080 to $139,275 (GS 14/15) per year, commensurate with qualifications.

Qualifications: A bachelor's degree (or higher) is required. Desirable applicants will have an advanced degree and demonstrated experience in conducting research and leading research teams in environmental health, toxicology, biology, physical science, mathematical statistics, or a related field.

How to Apply: Applicants should apply through Ezhire at **http://www.epa.gov/ezhire** Select apply for jobs. If you are already registered in Ezhire@EPA system, access the vacancy announcement through Registered Users. Otherwise, select New Users and complete the registration process. The vacancy announcement will be open through March 13, 2006. Application materials must be submitted with 48 hours from the closing date of the announcement. You need to submit the additional documentation described in the full text vacancy. Questions regarding this vacancy may be directed to **Joann Kelleher, Human Resources Management Division** at **kelleher.joann@epa.gov**.

The US EPA is an Equal Opportunity Employer.

UNIVERSITY OF DELAWARE

One of the oldest institutions of higher education in this country, the University of Delaware today combines tradition and innovation, offering students a rich heritage along with the latest in instructional and research technology. The University of Delaware is a Land-Grant, Sea-Grant, Urban-Grant and Space-Grant institution with its main campus in Newark, DE, located halfway between Washington, DC and New York City. Please visit our website at: **www.udel.edu.**

Assistant or Associate Professor, Ruminant Biology

Department of Animal and Food Sciences

12-month tenure-track appointment, 70% research, 30% teaching. The individual must develop an innovative, extramurally-funded, research program in ruminant biology that will complement the research of existing faculty in ruminant nutrition, forage production and ruminant health/production medicine. Potential areas of research include metabolism, anaerobic ruminant microbiology, toxicology, animal health, molecular biology, genomics, food safety, or environmental issues. An understanding of, or experience in, commercial production practices of ruminant animals is desirable. The individual will be responsible for undergraduate instruction and academic advisement, and participation in the undergraduate research program.

Submit a letter of application, current curriculum vitae, statement of teaching philosophy and research plans, and three references to Lesa Griffiths, 186 S. College Ave., University of Delaware, Newark, DE 19716-1440. Application deadline is April 15, 2006.

The UNIVERSITY OF DELAWARE is an Equal Opportunity Employer which encourages applications from Minority Group Members and Women.

Department of Health and Human Services
National Institutes of Health
National Institute on Aging

The National Institute on Aging, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS) is recruiting for **four post-doctoral fellows** in the Laboratory of Genetics, Intramural Research Program (IRP):

1) with a background in cell based screens or imaging studies to work in the Image Informatics and Computational Biology Unit (IICBU), for high-throughput automated visual screening of RNAi libraries. The interdisciplinary group has developed image classification algorithms based on machine learning techniques, and we would like to apply these to the systematic reconstruction of genetic pathways. For additional information on this research, please go to: **(http://www.grc.nia.nih.gov/branches/lg/iicbu/iicbu.htm)**. Applicants should send the curriculum vitae, via email to Dr. Ilya Goldberg at, **goldbergil@grc.nia.nih.gov**.

2) with a background in biochemistry to work in the Transcription Regulation and Remodeling Section (TRRS), on purification of multi-protein complexes and analysis of their structures and functions **(http://www.grc.nia.nih.gov/branches/lg/trru/trru.htm)**. Projects include studies of chromatin-remodeling mechanisms (*G&D 19:1662-7*), DNA damage response, and human genomic instability diseases (*Nat. Genet. 35:165-170; 37: 958-63*). Applicants should send the curriculum vitae, via email to Dr. Weidong Wang, **wangw@grc.nia.nih.gov**

3) with a background in mouse development to work in the Developmental Genomics and Aging Section, to conduct the study of preimplantation mouse development (*Dev. Cell 6: 117-131, 2004*) and embryonic stem cells (*PLoS Biol. 1: 410-419, 2003*). The work utilizes embryogenomics approaches (*Trends Biotechnol. 19: 511-518, 2001*) and focuses on the identification and characterization of genes that are critical for the maintenance of pluripotency and/or for early commitment to different cell lineages. Applicants should send the curriculum vitae via email to Dr. Minoru Ko, **kom@grc.nia.nih.gov**.

4) with a background in molecular genetics to work in the Human Genetics Section, on the determination of skin appendage formation in vitro, based on signaling pathways operating with the EDA (ectodysplasin) TNF-ligand (*Hum. Molec. Genet. 11:1763-1773; Hum. Molec. Genet. 12: 2931-2940*). The aims include the understanding of how hair follicles form, as a model system for both development and possible regeneration. Approaches include histology, keratinocyte cell differentiation, and immunocytochemistry, as well as a range of genomic and physiological techniques. Applicants should send the curriculum vitae, via email to Dr. David Schlessinger, **schlessingerd@grc.nia.nih.gov.**

The successful individuals will possess an M.D. or Ph.D. degree in biochemistry, molecular genetics or a related field, with no more than five years of Post Doctoral research experience. Salary is commensurate with research experience and accomplishments.

DHHS and NIH are Equal Opportunity Employers

Harvard School of Public Health

Assistant Professors of Immunology and Infectious Diseases

The Department of Immunology and Infectious Diseases at the Harvard School of Public Health seeks candidates for up to three positions as assistant professor. These positions are on the tenure ladder.

Each of the successful candidates will be expected to develop an independent research program on the immunology and biology of infectious disease(s) important to developing countries (e.g., malaria, TB, AIDS). A focus on vaccine and drug discovery is desirable. S/he will teach at the graduate level and will direct doctoral students in their dissertation research.

Applicants should have a doctoral degree and postdoctoral research experience in a relevant area.

Please send a letter of application, including a statement of current and future research interests, a curriculum vitae, sample publications, and the names and addresses of three referees to the following address. Applicants should ask their three referees to write independently to this address: **Chair, Assistant Professor Search Committee, c/o Robert Brier, Department of Immunology and Infectious Disease, Harvard School of Public Health, 665 Huntington Avenue, Room 813, Boston, MA 02115; bbrier@hsph.harvard.edu.**

Harvard University is committed to increasing the number of women and minorities in its faculty, and encourages applications from such candidates.

Leslie Dan Faculty of Pharmacy, University of Toronto
Canada Research Chair in Molecular Medicine/Molecular Pharmacology

Applications are invited for a tenure-stream position at the rank of Assistant, Associate or Full Professor in the area of Molecular Medicine/Molecular Pharmacology. The successful candidate will be expected to establish an independent and externally funded program of research into the nature and determinants of drug action, to supervise graduate students and to teach at the graduate and undergraduate levels. Upon appointment, he or she will be nominated for a Canada Research Chair at the appropriate level (Tier 1 or Tier 2). Canada Research Chairs have been established by the Government of Canada to foster research of the highest quality at Canadian universities (**http://www.chairs.gc.ca**).

We seek an individual whose research is directed toward questions of structure and mechanism at the molecular level. Signaling processes are an area of interest within the department, and specific topics could include neurotransmitter and hormone receptors, ion sensors and other drug targets or signaling elements. We encourage applicants who plan to employ a combination of biochemical, biophysical, computational or imaging approaches. While research focus is a consideration, all applications will receive close attention. The selection will be based in large measure on the originality and quality of recent and proposed work.

The University of Toronto and its affiliated teaching hospitals and research institutes maintain a strong presence in the physical, biological and medical sciences. Collaboration is encouraged and supported through cross-appointments, joint courses and various multidisciplinary programs. The Leslie Dan Faculty of Pharmacy offers excellent facilities for both teaching and research, and it is a partner in the Terrence Donnelly Centre for Cellular and Biomolecular Research (**http://ccbr.med.utoronto.ca**). The Faculty is presently undergoing an expansion that has included a substantial increase in research and in enrolment within its Graduate Department of Pharmaceutical Sciences (**http://www.utoronto.ca/pharmacy/graduate**). To accommodate this expansion, the Faculty will move to a new building in mid 2006 (**http://www.greatspaces.utoronto.ca/projects/pharmacy.htm**).

Interested individuals should forward a covering letter of application, a curriculum vitae, a 2 – 3 page statement of research plans, and three letters of reference to: **The Chair, Search Committee, Leslie Dan Faculty of Pharmacy, University of Toronto, 19 Russell St., Toronto, Ontario, Canada M5S 2S2.** Applications by email are encouraged; they should be addressed to **phmsearch@utoronto.ca**, please use Microsoft Word (doc) or Adobe Acrobat (pdf) format. Evaluation of applications will begin **March 17, 2006**.

The University of Toronto is strongly committed to diversity within its community. The University especially welcomes applications from members of visible minority groups, women, Aboriginal persons, persons with disabilities, members of sexual minority groups and others who may contribute to further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

CHAIR
Biological Sciences Department
California State Polytechnic University, Pomona

The Biological Sciences Department at California State Polytechnic University, Pomona (Cal Poly Pomona) invites applications for the position of Chair beginning September 2006. We seek a scholar with a vision for promoting excellence in undergraduate and graduate teaching, research and other scholarly activities, as well as service. Candidates should have outstanding leadership and interpersonal skills, a record of securing extramural funding, demonstrated distinction in teaching, and a significant record of department, college and university service. The successful candidate will be expected to articulate and implement a vision for expanding faculty research productivity in a way that enhances our educational programs.

Cal Poly Pomona, one of the 23 California State University campuses, is a comprehensive Master's level university with a student body that is among the most diverse in the country. The Biological Sciences Department strives for excellence in both research and teaching, and places great value on the synergy between faculty scholarship and teaching. Faculty members maintain active research programs that provide hands-on learning opportunities for undergraduate and graduate students. The Department has 37 tenure-track faculty, 6 lecturers, 40 teaching associates, and 9 support staff, and offers six undergraduate majors to nearly 1,000 students. A strong Master's program supports the scholarly activities of nearly 100 graduate students.

The successful candidate must have a distinguished record that would merit appointment at the rank of Full Professor with tenure. Applicants should forward (1) *curriculum vitae*, (2) statements on administrative, research, and teaching philosophy, (3) representative publication reprints, and (4) three letters of reference to: **Chair, Biology Chair Search, Biological Sciences Department, California State Polytechnic University, 3801 West Temple Avenue, Pomona, CA 91768-4132.** Review of applications begins on **April 1, 2006**. For further information, please visit the Department web site at: **http://www.csupomona.edu/~biology**.

California State Polytechnic University, Pomona is an Equal Opportunity, Affirmative Action Employer. Cal Poly Pomona subscribes to all state and federal regulations and prohibits discrimination based on gender, race, sexual orientation, national origin, disability, marital status, age, religion, or covered veteran's status.

CASE
CASE WESTERN RESERVE UNIVERSITY

NCI CCC
A Comprehensive Cancer Center Designated by the National Cancer Institute

Associate Director for Basic Research
Case Comprehensive Cancer Center

The NCI-designated Center coordinates cancer research of 270 members at Case, University Hospitals of Cleveland and The Cleveland Clinic. Nine Scientific Programs supported by 15 shared resources generate more than $110 million in direct annual research and manage 300 clinical trials with over 7,500 new cancer patients annually. Details at **http://cancer.case.edu**

Duties of the Associate Director: Advise the Director on all aspects of cancer research among Center members including: Scientific Program organization and membership, recruitment of basic laboratory based investigators, promotion of interdisciplinary and translational research in cancer genetics, signaling, DNA repair, tumor therapeutic targets, drug discovery, and disease based research; multi-investigator RFAs, etc., oversee pilot grant applications, reviews and awards, advise new initiatives' fund allocation, share oversight of current and new cores, participate in Senior Leader and Scientific Program meetings.

Candidates must have qualifications for associate professor or higher appointment with tenure at Case, a primary appointment in a basic science department, active NIH funded cancer research, study section and training experience, multi-investigator research, and national recognition in their field.

Please send resume or inquiry to:

Stanton L. Gerson, MD, Director
stanton.gerson@case.edu • 216-844-8562
and/or
John B. Lowe, MD, Chair, Department of Pathology
john.lowe@case.edu • 216-368-3611

Case is an Affirmative Action/Equal Opportunity Employer. Minorities and women are encouraged to apply.

Assistant /Associate Professor in Pharmacokinetics
University of Toronto

Applications are invited for a tenure-stream position at the rank of Assistant /Associate Professor in the area of Pharmacokinetics, PK-PD modeling, Clinical Pharmacology or Biopharmaceutics. Candidates must hold a Ph.D. or an equivalent degree and have post-doctoral research experience. The successful candidate is expected to establish an independent, externally funded research program, to supervise graduate students, and to teach Pharmacokinetics at the graduate and undergraduate levels. We seek individuals whose research interests complement existing strengths within the Faculty. While some areas are of particular interest, the selection will be based primarily on the originality and quality of recent and proposed research. Related areas of investigation within the Faculty include drug transport, drug metabolism, metabolite kinetics, radioimaging, drug targeting, and delivery systems.

The University of Toronto and its affiliated teaching hospitals and research institutes maintain a strong presence in the physical, biological, and health-related sciences. The Leslie Dan Faculty of Pharmacy offers new, state-of-the-art facilities (**www.greatspaces.utoronto.ca/projects/pharmacy.htm**), and is a partner in the Terrence Donnelly Centre for Cellular and Biomolecular Research (**http://ccbr.med.utoronto.ca**). The Faculty is presently undergoing an expansion that has included a substantial increase in research faculty and in enrolment within its Graduate Department of Pharmaceutical Sciences (www.utoronto.ca/pharmacy/graduate). Collaboration is encouraged and supported through cross-appointments, joint courses, and multidisciplinary programs. Opportunities also exist for interactions with pharmaceutical companies in the Toronto area.

Interested individuals should forward a covering letter of application, a curriculum vitae, a 2 - 3 page statement of research plans, and three letters of reference to: **The Chair, Search Committee, Leslie Dan Faculty of Pharmacy, University of Toronto, 19 Russell St., Toronto, Ontario, Canada M5S 2S2.** Applications by email are encouraged; they should be addressed to **phmsearch@utoronto.ca**, please use Microsoft Word (doc) or Adobe Acrobat (pdf) format. Evaluation of applications will begin **April 17, 2006**.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups and others who may contribute to further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

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ALS SLA

Amyotrophic Lateral Sclerosis Society of Canada

The ALS Society of Canada seeks a Director of Research who will lead the development and implementation of a national ALS research strategy focusing on understanding the cause of and cure for Amyotrophic Lateral Sclerosis (ALS).

Qualifications required: PhD in a biological science - preference given to neuroscience and minimum five years' experience with an organization closely aligned with and focused on scientific and medical advancements.

For full details on this position go to **www.als.ca/researchdirector**

Please submit resumes to David S. Cameron, National Executive Director, ALS Society of Canada at dc@als.ca by March 18, 2006.

FACULTY POSITION
CENTER FOR MICROBIAL PATHOGENESIS

The CENTER FOR MICROBIAL PATHOGENESIS at Columbus Children's Research Institute, Columbus Children's Hospital, and the Department of Pediatrics, College of Medicine and Public Health, The Ohio State University seek PhD, MD, or MD/PhD candidates for tenure-track positions at the Associate or Full Professor rank to develop and conduct independent research programs in the fields of cellular and molecular microbiology as well as innate and acquired immunity. Areas of emphasis include bacterial pathogens causing disease in the respiratory and genitourinary tracts. Research space is available within the Columbus Children's Research Institute. The recruitments are part of a larger 5 year planned expansion of research initiatives by the institution and include the recruitment of 40 new investigators and the recent construction of a new 160,000 square foot five-story research building that houses 48 state-of-the-art laboratory modules, completed in January of 2004. The Institute is equipped with a state-of-the-art mouse facility and DNA sequencing, flow cytometry, informatics, histopathology, transgenic, microarray, ES cell, and transgenic cores. Joint appointments within graduate departments of The Ohio State University are available.

For more information, please visit our website at **www.ccri.net**.

Address correspondence with three references and curriculum vitae to:

Lauren O. Bakaletz, Ph.D.
Director, Center for Microbial Pathogenesis
Columbus Children's Research Institute
700 Children's Drive
Columbus, OH 43205
Phone: (614) 722-2915 FAX: (614) 722-2818
E-mail: bakaletl@ccri.net

The Ohio State University is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and individuals with disabilities are encouraged to apply.

THE OHIO STATE UNIVERSITY

COLUMBUS Children's RESEARCH INSTITUTE
Supporting The Mission Of Columbus Children's Hospital, Inc.

FELLOWSHIPS

Transatlantic Biotechnology Exchange Fellowship Opportunity (Call for proposals): The US-EU Task Force on Biotechnology Research announces support for short-term exchanges of US scientists with interest in collaborating with EU laboratories. The exchanges are designed to foster transatlantic collaborative interactions and enhance the professional development of the exchange fellows through the acquisition of expertise in microbial environmental biotechnology. The fellowship will support visits from one to six months in a host EU laboratory, including travel, housing and a living supplement. Salary for the exchange fellow, institutional bench fees, and routine laboratory supplies are **NOT** covered. The exchanges are intended to foster the development of early career scientists who are US citizens or permanent residents, but **any US scientist displaced from their duties by natural disasters such as hurricanes Katrina or Rita are especially encouraged to apply**. Other scientists who have initiated their predoctoral studies or have been awarded the Ph.D. degree no more than seven years from the application date may apply. Applicants should submit a one-page description of the research/training plan for the exchange, a budget with justification, a c.v. and letters of support from their immediate supervisor and the exchange host institution or scientific mentor. All components of the application as well as electronic contact information for both the Ph.D. mentor and host should be submitted in the form of e-mail to **Dr. Joseph Suflita** (Univ. Oklahoma; **jsuflita@ou.edu**) or to **Dr. Judy Wall** (Univ. Missouri; **wallj@missouri.edu**). Applications will be screened as received as funds allow. More information can be obtained at **http://www.biochem.missouri.edu/EU-US-BiotechFellow**.

Premier's Research Chair in Biomaterials and Transportation
Regular Full-Time Faculty
Department of Plant Agriculture – University of Guelph

OAC and the Department of Plant Agriculture are seeking an internationally-recognized Senior Research Chair to lead an emerging research program in the area of biomaterials and transportation at the University of Guelph. The successful candidate should have documented strength within and between plant biology and materials engineering. They will provide leadership at the interfaces of chemical and structural engineering, agricultural chemistry, plant biology, and traditional and molecular plant breeding. (S)he will complement strengths in material science and engineering at partnering institutions and will be expected to build a network of collaborators that will place Ontario at the fore front of discovery and innovation in this exciting new field. Applicants should have demonstrated expertise in Biochemical Engineering, Biological Material Sciences, Bio-product Development or a related discipline. The incumbent will liaison with the automotive and materials industries and with university, provincial and federal government colleagues to develop research directions that effectively integrate agricultural and forestry-based raw materials into cost-effective, performance enhanced consumer products with emphasis on the automotive sector. This faculty position is 90% research/extension and 10% teaching (graduate level). The successful candidate will have a Ph.D. and a strong track record in biopolymer chemistry, biomaterials science or related fields.

Candidates should submit a curriculum vitae and arrange to have three letters of reference sent to:

Dr. Gary Ablett, Chair
Department of Plant Agriculture
University of Guelph • Guelph, Ontario N1G 2W1
gablett@uoguelph.ca • Fax (519) 821-8660

APPLICATION DEADLINE: March 31, 2006 or until a successful candidate is identified.

The University of Guelph is committed to an employment equity program that includes special measures to achieve diversity among its faculty and staff. We therefore particularly encourage applications from qualified aboriginal Canadians, persons with disabilities, members of visible minorities and women.

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

UNIVERSITY of GUELPH

Faculty Position in Marine Science

The University of Southern Mississippi's Department of Marine Science (DMS) announces the availability of a tenure-track faculty position at the rank of Assistant Professor beginning July 1, 2006 (beginning of the 2006 academic year). We seek applications in the area of remote sensing of oceanic and coastal waters. The successful candidate is expected to have a research program with a strong focus on coastal and oceanic variability using various sensors and platforms (e.g., SeaWiFS, MODIS, hyperspectral sensors, LIDAR, satellite and airborne). Demonstrated expertise and familiarity with radiative transfer and optical theory are desirable. Teaching duties will include graduate teaching and course development in ocean remote sensing and related areas. Individuals who can complement the department's existing strengths, collaborate with other research organizations at the Stennis Space Center, and take advantage of the growing community of expertise on the Gulf Coast in LIDAR coastal surveying and LIDAR/Hyperspectral data fusion are especially encouraged to apply. DMS is a vibrant, rapidly developing, multidisciplinary academic unit with research and teaching programs in biological, geological, and physical oceanography, marine chemistry, and hydrographic science. The department is located at the John C. Stennis Space Center on the Mississippi Gulf Coast, which is home to more oceanographers and hydrographers than any other location in the world. In addition to NASA research activities, the Stennis Space Center is also home to the Naval Research Laboratory (NRL-SSC), the U.S. Naval Oceanographic Office, NOAA's National Data Buoy Center, the NOAA Coastal Data Development Center, and the Maury Oceanographic Library, one of the country's premier oceanographic libraries. Detailed information about the department is available at **www.usm.edu/marine**.

Applicants must submit, preferably by electronic mail, a curriculum vitae with a research plan; a statement of teaching philosophy; and names, mailing addresses, and e-mail addresses of four references to: **Dr. Stephan D. Howden, The University of Southern Mississippi, Department of Marine Science, 1020 Balch Boulevard, Stennis Space Center, MS 39529; Stephan.Howden@usm.edu**. Review of applications will begin **March 15, 2006** and will continue until position is filled.

OHSU | OGI SCHOOL OF SCIENCE & ENGINEERING

Gordon and Betty Moore Endowed Chair in Biomedical Engineering

The OGI School of Science and Engineering at Oregon Health & Science University seeks candidates for an endowed Professorship. Applications are invited from distinguished scientists working in health-related areas of nanobiotechnology, as broadly defined. Applicants should have a record of significant academic accomplishment; preference will be given to those candidates with strong records of extramural funding, program development, and translational research.

The successful candidate will exhibit leadership skills, including the ability to recruit and mentor promising young faculty. He/she will be expected to build a program that will engage OHSU scientists and clinicians, state and regional organizations, and industrial partners. All areas of research will be considered; existing faculty strengths include neuroscience, cardiovascular disease, biomedical optics and imaging.

The Biomedical Engineering (BME) Department will soon move to a new building near the Willamette River in downtown Portland, Oregon. This location, connected to OHSU's main campus by an aerial tram, will anchor OHSU's emerging South Riverfront Campus — a focus for biotechnology development in Portland that will integrate OHSU and other regional academic, research and clinical activities.

Applicants should submit electronically a curriculum vitae, a statement of research and teaching interests and the names of six potential references to: **moore.chair.search@bme.ogi.edu**. Inquiries and requests for additional information may be sent to: **moore.inquiries@bme.ogi.edu**. All contacts will be held in confidence.

OHSU is an Affirmative Action, Equal Opportunity Institution

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MAYO CLINIC

Postdoctoral Scientist Positions in Pancreatic Cancer Research Rochester, Minnesota, U.S.A

Scientists affiliated with the Mayo Clinic Specialized Program of Research Excellence (SPORE) in Pancreatic Cancer wish to fill postdoctoral positions, available immediately. Highly motivated, talented, career-oriented candidates are urged to apply.

- RAS signal transduction in human pancreatic cancer cells and mouse pancreatic cancer models. A strong background and considerable experience in molecular genetics, developmental biology, protein-protein interaction, and siRNA-based technique is required for this position. Experience in model organisms and mouse genetics helpful.
- Mitotic regulation and cancer biology. The lab currently focuses on understanding the roles of the tumor suppressor BRCA2 and several novel mitotic regulators in pancreatic cancer. Experience in molecular and cell biology and mouse genetics is required, and a strong background in molecular and cellular biology is essential.
- Apoptotic signaling and its regulation in human pancreatic carcinoma cells with an emphasis on reversal of drug resistance. Strong background and experience in molecular and cell biology, including siRNA-based technique, is required.

Send curriculum vitae, summary of accomplishments, and the names of three references to: **Gloria M. Petersen, Ph.D., Principal Investigator, Mayo Clinic SPORE in Pancreatic Cancer, Mayo Clinic, 200 First Street SW, Rochester, Minnesota, 55905, USA.**

ALBERT-LUDWIGS-UNIVERSITÄT FREIBURG

The following position will be available at the **Department of Radiotherapy, University Hospital Freiburg,** Germany, from the October 1st 2007

W 3-Professor for Radiotherapy (Chair)
(Succession of Professor H. Frommhold)

The future holder of the Chair (male/female) will represent this field in research, teaching and patient care. He/she will also be the Head of the Department of Radiotherapy. The internationally accomplished candidate should have expertise in all scientific, clinical, and practical concerns. Readiness to collaborate with other clinical and theoretical disciplines of the university hospital and other scientific institutions is mandatory. The successful candidate is expected to strengthen the scientific main foci of the Medical Faculty, especially the research area of "Oncology and Functional Genetics", and to participate in the Comprehensive Cancer Center Freiburg (CCCF). An M.D. degree, a board certification in Radiotherapy and a postdoctoral lecturing qualification (Habilitation) or the equivalent are required. The candidate must have outstanding credentials in research, teaching, and patient care. He/she must have impeccable interpersonal, managerial and supervisory skills as well as significant experience in leading clinical, educational and research programs. Applicants might be asked to demonstrate their teaching skills.
The Faculty of Medicine and the University Hospital intend the implementation of a center structure. This might affect the interaction of existing divisions as well as the structural integration of individual professors. The position is tenured. However, in accordance with the State University Law (LHG), if this is the candidate's first appointment to a professorship, employment will be initially limited to four years with prolongation subject to evaluation. Exceptions are possible, particularly in the case of applications from abroad or from a non-university area. The duties and responsibilities in the field of patient care will be subject to a separate employment contract with the University Hospital Freiburg.
The University of Freiburg is an equal opportunity employer. Applications of women are especially invited. Handicapped candidates with equivalent qualifications will be given preference as well.
For application forms please apply to the Dean of the Medical Faculty (Phone: ++49 761 270 7235/7234; e-mail: dekanat-professuren@uniklinik-freiburg.de). Completed applications along with all pertinent documents should be sent to the Dean of the Medical Faculty, Prof. Dr. med. Ch. Peters, Albert-Ludwigs-University, D-79085 Freiburg, no later than April 13th, 2006.

Postdoctoral Fellow
Pathology and Laboratory Medicine
Emory University School of Medicine

Postdoctoral positions are available at Emory University focusing on innate immunity of mucosal surfaces and regulation of epithelial barrier function/tight junctions. Opportunities exist for involvement in exciting projects aimed at understanding how leukocytes interact with epithelial cells with special emphasis on cell-cell adhesion/integrins, signaling, transmigration and epithelial barrier function. The role of junctional adhesion molecules and signal regulatory proteins in the above processes are actively being studied. A doctoral degree, experience in molecular/cell biology and protein biochemistry of eukaryotic systems and strong English communication skills are required. Preference will be given to individuals with previous experience in the fields of innate immunity and/or biology of epithelial cells. The successful applicant will join our epithelial pathobiology group comprised of six principal investigators with common interests that occupy new, fully equipped and interconnected research space.

Interested individuals should send resume to: **Dr. Charles Parkos, Emory University, Whitehead Biomedical Research Building, Room 105B, 615 Michael St. Atlanta GA 30322. email: cparkos@emory.edu**.

Emory University is an Equal Opportunity/ Affirmative Action Employer.

Canada Research Chair, Pharmaceutics and Pharmaceutical Chemistry
Leslie Dan Faculty of Pharmacy
University of Toronto

Applications are invited for a tenure-stream position at the rank of Associate or Full Professor in the areas of Pharmaceutics and Pharmaceutical Chemistry. We seek individuals whose scientific activities will complement existing strengths within the Faculty. While some areas are of particular interest, the selection will be based in large measure on the originality and quality of recent and proposed research. The successful candidate will be expected to establish an independent and externally funded research program, to supervise graduate students and to teach at the graduate and undergraduate levels. Upon appointment, the successful candidate will be nominated for a Canada Research Chair (www.chairs.gc.ca). Canada Research Chairs have been established by the Government of Canada to foster research of the highest quality at Canadian universities.

The University of Toronto and its affiliated teaching hospitals and research institutes maintain a strong presence in the physical, biological and medical sciences. Collaboration is encouraged and supported through cross-appointments, joint courses and various multidisciplinary programs. Opportunities also exist for interactions with pharmaceutical companies in the Toronto area. The Leslie Dan Faculty of Pharmacy offers excellent facilities for both research and teaching, and it is a partner in the Terrence Donnelly Centre for Cellular and Biomolecular Research (**http://ccbr.med.utoronto.ca**). The Faculty is presently undergoing an expansion that has included a substantial increase in research and in enrolment within its Graduate Department of Pharmaceutical Sciences (**www.utoronto.ca/pharmacy/graduate**). To accommodate this expansion, the Faculty will move to a new building in the Spring of 2006 (**www.greatspaces.utoronto.ca/projects/pharmacy.htm**).

Interested individuals are asked to forward a covering letter of application, a curriculum vitae, a statement of current and proposed research, a summary of teaching experience and philosophy, and three letters of reference to: **The Chair, Search Committee, Leslie Dan Faculty of Pharmacy, University of Toronto, at phmsearch@utoronto.ca**. Please apply electronically, using Microsoft Word or Adobe Acrobat (pdf) format. Letters of reference may be sent electronically or addressed to the above **Committee at 19 Russell St., Toronto, Ontario, Canada M5S 2S2**. Evaluation of applications will begin on **March 24, 2006**, and applications will be considered until the position is filled.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups and others who may contribute to further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

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POSITIONS OPEN

CARDIOVASCULAR FACULTY POSITION

Applications are invited for a faculty position at the Cardiovascular Research Institute (CRI) directed by **Dr. A. Martin Gerdes** at the South Dakota Health Research Foundation (SDHRF), **website: http://www.sdhrf.org,** in Sioux Falls. This position will be filled at the **ASSISTANT, ASSOCIATE,** or **PROFESSOR** level. Currently, the CRI has seven faculty engaged primarily in studies of the molecular mechanisms of heart failure. Applicants with a background in vascular disease, diabetes, heart failure, or heart development are encouraged to apply. Applicants must have a Ph.D./M.D. or equivalent degree and a minimum of two years of postdoctoral experience. Competitive salaries, startup funds, and laboratory space will be provided. The new faculty member will have access to CRI Physiology, Imaging, Cell Culture, and Molecular Biology Cores. The SDHRF is a partnership between the Sanford School of Medicine of the University of South Dakota and Sioux Valley Hospital and Health Systems in Sioux Falls. Sioux Falls is a rapidly growing, affordable city of approximately 140,000 with excellent schools, low crime, low taxes, and excellent health care.

Application directions: Applicants should submit a letter of interest, curriculum vitae, and three letters of reference.

Application deadline: Applications will be accepted until the position is filled. Review of applications will begin on March 15, 2006. Submit to:

Betty Poppens, Ed.D., Director of Human Resources
South Dakota Health Research Foundation
1100 E. 21st Street, Suite 700
Sioux Falls, SD 57105
E-mail: bpoppens@usd.edu.

PHARMACEUTICAL SCIENCES FACULTY POSITIONS
University of Southern Nevada
College of Pharmacy
South Jordan, Utah Campus

The University of Southern Nevada College of Pharmacy is establishing an extension program in the Salt Lake City, Utah area. The College is currently seeking applicants for full-time Faculty positions at the Utah campus in the areas of biochemistry, immunology, medicinal chemistry, pharmaceutics and pharmacology. Responsibilities include teaching in blocks and electives related to your discipline, participating in research and scholarly endeavors, and providing service to the College and the community.

Minimum requirements include a Ph.D. degree in biochemistry, immunology, medicinal chemistry, pharmaceutics, pharmacology, or any closely related discipline. The successful candidates should have excellent written and oral communication skills and an interest in teaching, especially creative and non-traditional methods of teaching. Documented evidence of research and other forms of intellectual initiatives are required. Recent Ph.D. graduates are welcome to apply. Salary and rank will be commensurate with qualifications and experience.

Candidates should submit a letter of interest, curriculum vitae, statement of educational philosophy, and contact information for three professional references to: **Thomas Metzger, Ph.D., University of Southern Nevada College of Pharmacy, 11 Sunset Way, Henderson, NV, 89014; e-mail: tmetzger@usn.edu.**

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RESEARCH DIRECTOR

The California Department of Food and Agriculture (CDFA) is seeking a motivated, innovative outside consultant to serve as Research Director for the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. Please refer to contract number RFP 05-0616 at **website: https://www.cscr.dgs.ca.gov/cscr/** for more information. Contact: **Ms. Joy Mountjoy (e-mail: jmountjoy@cdfa.ca.gov; telephone: 916-651-8182).** Deadline: March 30, 2006.

POSITIONS OPEN

FACULTY POSITIONS
Cellular Physiology
Integrative Physiology
University of Pennsylvania
School of Medicine
Department of Physiology

The Department of Physiology at the University of Pennsylvania's School of Medicine seeks candidates for several Assistant or Associate Professor positions on the tenure track. Rank will be commensurate with experience. The successful applicant will have experience in the field of cellular or integrative physiology with a focus on physiology, cell biology, and/ or structural biology. Responsibilities include conducting an independent research program on aspects of cellular and/or organismal physiology. The position also will involve training and teaching of graduate and/or medical students. Applicants must have an M.D. and/or Ph.D. or equivalent degree and have demonstrated excellence in research. The successful candidate will have experience involving biochemical, physiological, cell biological and/or structural biological approaches to problems of significance to integrated physiology of cells and organisms. Please submit curriculum vitae, a brief statement of research interests, and the three reference names to: **Physiology Search Committee, University of Pennsylvania, Department of Physiology, B400 Richards Building, 3700 Hamilton Walk, Philadelphia, PA 19104-6085. E-mail: emartin@mail.med.upenn.edu.** *The University of Pennsylvania is an Equal Opportunity, Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.*

POSTDOCTORAL FELLOWS
Medical College of Georgia

Postdoctoral fellowships are immediately available to study: (1) Role of G protein-couples receptors and their effectors in cancer cell growth and metastasis (**Y. Daaka,** *Science STKE* **re2**, 2004); and (2) Regulation of dynamin function in receptor mediated endocytosis and infectious particle uptake (**S. Ahn et al.,** *J.Biol. Chem* **277:** 26642, 2002); **G. Wang et al.,** *Proceedings of the National Academy of Sciences* epub, 2006). Successful candidates must have a Ph.D. and/or M.D. degree and working knowledge in molecular and cellular biology techniques. Please send curriculum vitae and names of three references to: **Dr. Yehia Daaka, Professor and Endowed Chair, Department of Pathology, Medical College of Georgia, BF-103, 1120 15th Street, Augusta, GA 30912,** or **e-mail: ydaaka@mcg.edu.** To apply online, see **website: http://www.mcg.edu/jobs/apply.**

The Medical College of Georgia is a Minority/Female/Veterans: Equal Employment Opportunity, Affirmative Action, and American with Disabilities Act Employer. E000081083.

ECOSYSTEM ECOLOGY
Carleton College

The Department of Biology invites applications for a temporary one-term position from 1 September 2006 to 30 November 2006. Teaching responsibilities include an upper-level course in ecosystem ecology with laboratory. Applicant should have Ph.D. degree or be near completion. Send letter of application, curriculum vitae, a brief statement of teaching and research interests, and three letters of reference to: **Professor Stephan Zweifel, Department of Biology, Carleton College, Northfield MN 55057-4025.** Application deadline is 13 March 2006. *Carleton is an Affirmative Action/Equal Opportunity Employer. We are committed to developing our faculty to better reflect the diversity of our student body and American society. Women and members of minority groups are strongly encouraged to apply.*

POSITIONS OPEN

PHYSIOLOGIST

The Department of Health Sciences at East Tennessee State University seeks applicants for a tenure-track position at the Assistant Professor level opening in August 2006. The position requires teaching undergraduate lecture and laboratory courses in anatomy/physiology and upper division human physiology. Development of a fundable research program is expected. Ph.D. preferred, however, candidates holding other graduate degrees with extensive university level teaching experience in physiology may be considered. Screening of applicants will begin in March 2006. Salary and rank will depend on qualifications and experience. Send curriculum vitae, the names, addresses, and telephone numbers of three references along with a letter of application addressing teaching philosophy and research interests to:

Dr. Michael T. Gallagher
Professor and Chair
East Tennessee State University
Department of Health Sciences
P.O. Box 70673
Johnson City, TN 37614-1709

East Tennessee State University is an Equal Opportunity/Affirmative Action Employer.

FOREST WILDLIFE LANDSCAPE ECOLOGIST. Assistant/Associate Professor, tenure-track, position 002-1042. For review of the full position announcement, refer to our **website: http://www.oregonstate.edu/jobs.** For additional information, contact: **John P. Hayes, Search Committee Chair, Department of Forest Science, 109E Richardson Hall, Oregon State University, Corvallis, OR 97331-5752. E-mail: john.hayes@oregonstate.edu.** To apply: For full consideration send a letter summarizing qualifications and vision for the position, curriculum vitae, copies of three publications, and the names and contact information for three references by March 6, 2006, to: **Ryan Hink, Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis OR 97331-5752.** *Oregon State University is an Equal Employment Opportunity/Affirmative Action Employer and is responsive to the needs of dual-career couples.*

CHILDREN'S HOSPITAL HARVARD MEDICAL SCHOOL

A **POSTDOCTORAL POSITION** is available to investigate the regulation of gene expression in the opportunistic pathogen Pseudomonas aeruginosa. Projects center on regulatory proteins that associate with the transcription machinery and on regulators of fimbrial gene expression. Candidates should have, or soon expect to receive, a Ph.D. with a strong background in molecular biology, microbiology, or biochemistry. Experience with P. aeruginosa desirable but not essential. Please submit a letter of interest, curriculum vitae, and names and contact information of three references to: **Simon L. Dove, Ph.D., Assistant Professor of Pediatrics, Children's Hospital, Division of Infectious Diseases, Enders 754, 300 Longwood Avenue, Boston, MA 02115. E-mail: simon.dove@childrens.harvard.edu.**

CHEMIST, ANALYTICAL needed with Bachelor's or foreign equivalent in biochemistry or chemistry and one year of experience to perform analytical testing of pharmaceutical finished products, raw material and stability samples, in-process and process validation samples using high performance liquid chromatography, UV-visible spectrophotometer, infrared spectroscopy (IR), wet chemistry and dissolution tester. Conduct analysis of finished products identification tests using thin layer chromatography and disintegration. Maintain documentation to ensure good laboratory practice and current good manufacturing practice compliance. Check analysis reports and documents. Mail resumes to: **Accumed Inc., 2572 Brunswick Pike, Lawrenceville, NJ 08648.** Job location: Lawrenceville, New Jersey. Ref ad 001, Human Resources Department. *Equal Opportunity Employer.*

POSITIONS OPEN

ASSOCIATE RESEARCH SCIENTIST

The Associate Research Scientist will be responsible for the studies in the laboratory related to the complications of diabetes in the kidney, as well as the impact of other injuries to the kidney that are directly toxic to glomerular cells. The applicant must be familiar with murine models of diabetes and nephropathy. The studies will include both in vivo and in vitro experiments mainly focused on cellular and molecular aspects of above renal diseases. The applicant must be expert in intravenous injections in mice; laser capture microdissection of glomerular tissue from mouse kidney; use of metabolic cages and the determination of functional changes in renal function.

Additional duties include: (1) laser capture microdissection of mouse kidney for isolation of glomerular tissue; (2) preparation and analysis of DNA and RNA; (3) real time PCR; (4) RNA isolation and real time polymerase chain reaction; (5)protein analysis (ELISA and WB); (6) histology, immunohisto(cyto) chemistry, and confocal microscopy; (7) cell isolation and culturing; (8) breeding and genotyping of transgenic mice.

The applicant must be able to perform these studies with minimal supervision.

E-mail resumes to **Karen Evans** at **e-mail: kme1@columbia.edu,** or mail resumes to: **Columbia University, College of Physicians and Surgeons, 630 W. 168th Street, P&S 17-401, New York, NY 10032, Attn: Karen Evans.**

Columbia University is an Affirmative Action/Equal Opportunity Employer.

POSTDOCTORAL FELLOW

Applications are being accepted for a Postdoctoral position on an NIH Training Grant at the Wake Forest University School of Medicine. This position will be effective July 1, 2007. A strong interest in sensory systems and multisensory integration is essential. A list of training grant faculty can be found on the **website: http://www1.wfubmc.edu/nba/Positions+Available/Training+Grant+Faculty.** For more information on the Department and areas of research emphasis, visit our **website: http://www.wfubmc.edu/nba/.** Candidates should send curriculum vitae, statement of research interest, and three letters of recommendation to:

Barry E. Stein, Ph.D.
Department of Neurobiology and Anatomy
Wake Forest University
School of Medicine
Winston-Salem, NC 27157-1010

This may also be sent via **e-mail: bestein@wfubmc.edu.** *Wake Forest University School of Medicine is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION available to study the role of the cytoskeletal linker protein, plakoglobin, in cardiac function (*Circ. Res.* **96:** 346, **97:** 474, 2005). Gene targeting in ES cells will be used to engineer a mouse model of Naxos disease. Experience with animal models is desirable with a strong background in molecular and cell biology. This is a collaborative project between the laboratories of **Drs. Glenn Radice and Vickas Patel, Cardiovascular Institute, University of Pennsylvania School of Medicine, Philadelphia, PA. E-mail: radice@mail.med.upenn.edu** or **vickas.patel@uphs.upenn.edu.**

POSTDOCTORAL POSITION to investigate mTOR signaling. Follow link **(website: http://www.healthsystem.virginia.edu/internet/pharmacology/faculty/pharmfaculty.cfm)** to Lawrence page for description of projects. Both foreign and domestic applicants at the Ph.D. level will be considered. Salary dependent on qualifications. To apply send a cover letter and resume by e-mail to **Dr. John C. Lawrence, e-mail: jcl3p@virginia.edu.** *The University of Virginia is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

University Of Nebraska Lincoln

POSTDOCTORAL RESEARCH ASSOCIATE POSITIONS are available at the **Nebraska Center for Virology in the School of Biological Sciences** at the **University of Nebraska, Lincoln,** to study molecular mechanisms of virus-host interactions and virus-induced oncogenesis. Candidates should have Ph.D. or equivalent, and experience in microbiology/virology, molecular biology, biochemistry, or cell biology. For more program information, visit **website: http://www.unl.edu/virologycenter/faculty/zhang.html.** To be considered for this position, go to **website: http://employment.unl.edu.** Review of applications will begin on March 17, 2006.

The University of Nebraska is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity. We assure reasonable accommodation under the Americans with Disabilities Act; contact J. Walker at ***telephone: 402-472-4560*** *for assistance.*

FACULTY POSITIONS, VIROLOGY AND BACTERIAL PATHOGENESIS. The Department of Microbiology at University of Texas (U.T.) Southwestern Medical Center is seeking new faculty at the **ASSISTANT OR ASSOCIATE PROFESSOR** (tenure track) levels. Faculty will be expected to develop front-rank, competitive, independent research programs in their chosen fields and contribute to the teaching of medical and graduate students. For virology candidates, emphasis on one or more aspects of the viral life cycle (host-pathogen interactions, viral pathogenesis, disruption of viral replication, command of host cell processes, viral immunology, et cetera) is desirable to complement existing strengths in HCV, West Nile virus, HIV/SIV, and viral oncogenesis, but research on any virus of medical relevance is of interest. For bacterial pathogenesis, areas of particular interest include STDs, emerging/re-emerging pathogens, cellular microbiology, host-pathogen interactions, select agents, and opportunistic infections, but all outstanding candidates are encouraged to apply. Attractive startup packages, including competitive salaries, and new laboratory space, are available to conduct research in an expanding, dynamic environment. For exceptional Assistant Professor candidates, an Endowed Scholars Program offers startup funds of $700,000 (plus $300,000 towards salary) over a four-year period. Candidates should have a Ph.D. and/or M.D. degree with at least three years of postdoctoral experience and an exceptional publication record. Candidates please forward curriculum vitae, three letters of recommendation, two or three representative publications, and a brief summary of future research to: **Dr. Michael V. Norgard, Chair, Department of Microbiology, University of Texas Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, TX 75235-9048 (fax: 214-648-5905; e-mail: michael.norgard@utsouthwestern.edu).** *U.T. Southwestern is an Equal Opportunity University.*

POSTDOCTORAL POSITION
Virginia Commonwealth University
School of Medicine

Postdoctoral positions are available to study Signaling in Tissue Injury and Repair in several well-funded laboratories at the Virginia Commonwealth University Medical Center in Richmond, Virginia. The positions are funded by an NIH Institutional National Research Service Award (T32 GM008695). The objective of this training program is to provide an intensive two-year laboratory experience for young Physicians and basic Scientists to help them develop as independent investigators in preparation for a career in academics. For additional information see **website: http://www.vcu.edu/biochem/department/pos-nrsa.shtml.** Please contact **Dr. Diegelmann** at **e-mail: rdiegelm@vcu.edu** for further information and requirements. Applicants must be willing to visit Richmond for an interview. *Virginia Commonwealth University is an Equal Opportunity Employer.*

POSITIONS OPEN

GEOLOGICAL OCEANOGRAPHER. Moss Landing Marine Laboratories (MLML) and **San Jose State University** (SJSU) announce a tenure-track position in geological oceanography at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR.** We are seeking a field-oriented Scientist with broad interests in geological oceanography and in collaborative research and education endeavors with colleagues at MLML and the Monterey Bay and central California marine science community. MLML is a multi-disciplinary marine science research and teaching institution. Research resources include a fleet of small and large research vessels. The applicant must have a strong commitment to quality instruction at the undergraduate/graduate level and pursue a vigorous research program involving M.S. students. A Ph.D. is required. Instructional duties will include a core course in geological oceanography and other courses related to the candidate's and students' interest. Applicants should have sensitivity to the educational goals of a multicultural student population. Further information is found at **website: http://www.mlml.calstate.edu.** Review of applications begins on April 1, 2006, and continues until the position is filled. Send a letter discussing teaching and research interests, professional accomplishments and goals, curriculum vitae, and contact information for at least three references to: **Dr. Kenneth Coale, Director, Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039.** *MLML/SJSU is an Equal Opportunity/Affirmative Action/Title IX Employer.*

TENURE-TRACK ASSISTANT OR ASSOCIATE PROFESSOR, NEUROPHARMACOLOGY
The Ohio State University College of Medicine, Columbus, Ohio
Department of Pharmacology

The Department of Pharmacology **(website: http://www.medicine.osu.edu/pharmacology)** expands its research program with a new tenure-track faculty position. We seek motivated applicants specializing in the areas of molecular neuropharmacology, systems pharmacology, neurogenetics and pharmacogenetics, or similar disciplines, with emphasis on neuroscience and interest in clinical translations for treatment of mental disorders. Applicants should have a Ph.D. or M.D./Ph.D. degree and subsequent experience demonstrating outstanding research potential, with commitment to excellence in graduate and medical student teaching, and a track record of extramural funding

Ongoing review of applications occurs from February 2006, until the position is filled.

Send curriculum vitae, statement of research goals, a selection of publications and three letters of recommendation to:

CNS Pharmacologist Search Committee
Department of Pharmacology
The Ohio State University College of Medicine
5072 Graves Hall
333 West Tenth Ave
Columbus, Ohio 43210
E-mail: Sherry.Ring@osumc.edu

Ohio State University is an Equal Opportunity, Affirmative Action Employer.

The University of Arizona, College of Medicine invites applications for **HEAD, DEPARTMENT OF IMMUNOBIOLOGY.** The University of Arizona College of Medicine invites applications and nominations for a position that entails a unique opportunity to serve as Head and work with the Dean and the faculty to formulate the direction for a new Department of Immunobiology. The competitive recruiting package includes an attractive salary, generous laboratory space, and resources for new faculty hires, equipment, personnel and operating expenses.

Details are available at **website: http://medicine.arizona.edu/search** or direct inquiries to **Dr. Marilyn Halonen** at: **e-mail: mhalonen@e-mail.arizona.edu.** Review will begin April 1, 2006. *The University of Arizona is an Equal Employment Opportunity/Affirmative Action, Minorities/Women/Persons with Disabilities/Veterans Employer.*

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Guidelines, application forms and information are available on the Cordis website: http://fp6.cordis.europa.eu.int/index.cfm?fuseaction=UserSite.FP6DetailsCallPage&call_id=266

For further information, please visit the Descartes website: http://europa.eu.int/comm/research/descartes/index_en.htm
or please contact: rtd-descartes@cec.eu.int

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Prof. Roger Jowell
City University, United Kingdom
Winner of the 2005 Descartes Research Prize

"The Descartes Prize is an enormously generous and prestigious prize, and I am deeply honoured to receive it, of course. But more than that, it provides a huge and very welcome boost to science communication and research -- two areas that are central to all our futures but are often underappreciated."
Bill Bryson
Writer, United Kingdom
Winner of the 2005 Descartes Communication Prize

The Descartes Prize for Excellence in Scientific Collaborative Research

Since the launch of this Prize in 2000, 16 different projects have been rewarded, involving 105 teams from 24 EU and non EU countries, which achieved major scientific breakthroughs in key European research areas.

In 2006, €1.15 million will be shared among winners and finalists. Up to five winning teams will be rewarded a minimum of €200,000 each and five finalist teams will receive €30,000 each.

The Prize recognizes Europe's most outstanding scientific and technological results achieved through cross-border collaborative research.

Proposals for the Research Prize can be made directly by the research teams from appropriate public and private organisations. Universities and foundations may also submit candidate teams.

The Descartes Prize for Excellence in Science Communication

Now in its third year, this Prize intends to stimulate interest in science communication and to improve the quality of science communication towards the general public. It rewards creative achievements in the fields of television, radio, publishing, public events, etc.

Since 2004, 10 European personalities have won this prize, selected by a panel of leading EU scientists and media professionals. This year, up to five winners will receive a minimum of €50,000 each and five finalists €5,000 each.

This prestigious competition targets organisations or individuals who have already been selected as winners by European and/or national organisations which carry out existing science communication prizes of any kind.

Submission of proposals shall be made by these prize-giving organisations.

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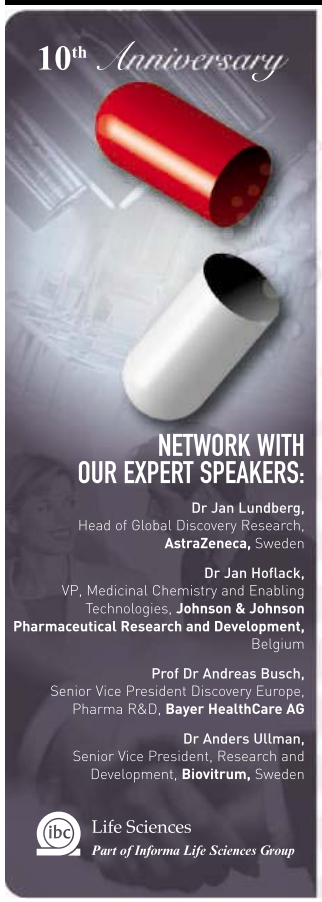

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Regulatory RNAs

May 31 - June 5, 2006

Abstract Deadline: March 8, 2006

Organized by Bruce Stillman & David Stewart

Topics:

- RNA Interference
- Micro RNAs
- Genome regulation by dsRNA
- Heterochromatin and RNA
- Post-transcriptional regulation
- Translational control by RNA
- Regulation of cell fate determination
- Novel ribonucleoprotein complexes
- Sense-antisense transcription
- Bacterial small RNAs
- Viral regulatory RNAs
- RNA guides
- Riboswitches
- Ribonuclease III superfamily
- Hidden transcriptome
- Applications of small RNAs in health & disease

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Partial List of Speakers:

David Barford, *Institute of Cancer Research, UK*
David Bartel, *HHMI/Whitehead Institute, MIT*
Brenda Bass, *HHMI/University of Utah School of Medicine*
David Baulcombe, *John Innes Centre, UK*
Rene Bernards, *Netherlands Cancer Institute*
Elizabeth Blackburn, *University of California, San Francisco*
Irene Bozzoni, *University of Rome "La Sapienza", Italy*
Gordon Carmichael, *University of Connecticut Health Center*
Richard Carthew, *Northwestern University*
Thomas Cech, *Howard Hughes Medical Institute*
Bryan Cullen, *HHMI/Duke University*
James Dahlberg, *University of Wisconsin Madison*
Robert Darnell, *HHMI/The Rockefeller University*
Jennifer Doudna, *University of California, Berkeley*
Gideon Dreyfuss, *HHMI/University of Pennsylvania*
Sean Eddy, *HHMI/Washington University*
Thomas Gingeras, *Affymetrix*
Susan Gottesman, *National Cancer Institute*
Rachel Green, *Johns Hopkins University School of Medicine*
Carol Greider, *Johns Hopkins University School of Medicine*
Shiv Grewal, *National Institute of Health*
Gregory Hannon, *Cold Spring Harbor Laboratory*
Edith Heard, *Curie Institute, France*
Oliver Hobert, *Columbia University College of P&S*
Alexander Huttenhofer, *University of Innsbruck, Austria*
Elisa Izaurralde, *EMBL Heidelberg, Germany*
Steve Jacobsen, *HHMI/University of California, Los Angeles*
Richard Jorgensen, *University of Arizona*
Leemor Joshua-Tor, *Cold Spring Harbor Laboratory*
V. Narry Kim, *Seoul National University, South Korea*
Mitzi Kuroda, *Harvard University*
Jeannie Lee, *Massachusetts General Hospital*
Robert Martienssen, *Cold Spring Harbor Laboratory*
Marjori Matzke, *Gregor Mendel Institute, Austria*
Danesh Moazed, *Harvard Medical School*
Timothy Nilsen, *Case Western Reserve University*
Renato Paro, *University of Heidelberg, Germany*
Dinshaw Patel, *Memorial Sloan-Kettering Cancer Center*
Norbert Perrimon, *Harvard Medical School*
Craig Pikaard, *Washington University*
Ronald Plasterk, *Hubrecht Laboratory, The Netherlands*
Scott Poethig, *University of Pennsylvania*
Nicholas Proudfoot, *University of Oxford, UK*
Gary Ruvkun, *Massachusetts General Hospital*
Peter Sarnow, *Stanford University*
Phillip Sharp, *Massachusetts Institute of Technology*
Frank Slack, *Yale University*
Nahum Sonenberg, *McGill University, Canada*
David Spector, *Cold Spring Harbor Laboratory*
Joan Steitz, *Yale University School of Medicine*
Gisela Storz, *National Institute of Child Health & Human Development*
Michael Terns, *University of Georgia*
Marja Timmermans, *Cold Spring Harbor Laboratory*
Sandra Wolin, *Yale University School of Medicine*
Phillip Zamore, *University of Massachusetts Medical School*
Qiang Zhou, *University of California, Berkeley*

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POSITIONS OPEN

FOREST ECOLOGIST. The Wilderness Society seeks an experienced Forest Restoration Ecologist, with an ability to work in the nexus between public policy and science, to join our regional conservation team's efforts to protect Montana's national forests. We seek an Applied Ecologist who can synthesize and analyze existing scientific information, develop new information, and translate these ideas into a format that will influence federal land management decisions in Montana. For a complete job description see the career page at **website: http://www.wilderness.org.** To apply please submit a cover letter explaining your qualifications for this position, resume, writing sample, along with names, addresses, and telephone numbers of three references to: **Ms. Geri Wardlow, The Wilderness Society, 1615 M Street, Washington DC 20036; fax: 703-327-0415; e-mail: geriw@tws.org.**

POSTDOCTORAL POSITIONS are available to study the role of homeobox genes in development and cancer. Qualified individuals should have a Ph.D and/or M.D., as well as prior experience in molecular biology, mouse genetics, and/or developmental biology. Please send curriculum vitae, summary of research interests, and three confidential letters of reference to: **Cory Abate-Shen, Ph.D., Center for Advanced Biotechnology and Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 679 Hoes Lane, Piscataway, NJ 08854. Fax: 732-235-5789. E-mail: abate@cabm.rutgers.edu.**

GRANT WRITER/ASSOCIATE RESEARCH SCIENTIST

Institute of Renewable Natural Resources seeks Grant Writer to facilitate and assist teams of faculty to pursue funding from federal agencies. M.S. required, Ph.D. and writing experience preferred. To apply, go online at **website: http://greatjobs.tamu.edu** using Vacancy 01653 by March 10, 2006.

POSITIONS OPEN

BEHAVIORAL ENDOCRINOLOGIST

The Department of Biological Sciences at Eastern Kentucky University invites applications for a Tenure-Track position in behavioral endocrinology at the Assistant Professor level beginning August 15, 2006. Additional information about the position and the application procedures are available on the department's website **(website: http://www.biology.eku.edu)** and from the Search Committee Chair:

Dr. Charles Elliott
Department of Biological Sciences
Eastern Kentucky University
Richmond, KY 40475
E-mail: charles.elliott@eku.edu

Eastern Kentucky University is an Equal Employment Opportunity/Affirmative Action institution that values diversity in its faculty, staff and student body. In keeping with this commitment, the University welcomes applications from diverse candidates and candidates who support diversity.

The Bioinformatics Research Center at North Carolina State University invites applications for a **TENURE-TRACK ASSISTANT PROFESSOR** with research emphasis in one or more of the following areas: genomics, proteomics, networks, and population genetics. The appointment will be in either the Department of Genetics or the Department of Statistics. All applicants must have a Ph.D. in genetics, statistics, or a related field. Postdoctoral research experience is preferred. To apply for this position, please visit our **website: http://jobs.ncsu.edu** (position 01-09-0601) and provide an application, curriculum vitae, and statement of research interests and arrange to have at least three letters of recommendation sent to **e-mail: cb_search@statgen.ncsu.edu,** or faxed to **fax: 919-515-7315. Website: http://www.bioinformatics.ncsu.edu.** *Affirmative Action/Equal Opportunity Employer. In addition, North Carolina State University welcomes all persons without regard to sexual orientation. ADA call telephone: 919-515-3148.*

POSITIONS OPEN

MEDICAL INFORMATION RESEARCHER/WRITER

PinnacleCare, a growing health management company based in Baltimore, Maryland is seeking a Medical Researcher/Writer. The successful candidate will perform information research mostly among biomedical journals to discover the latest findings in disease mechanism, diagnosis, current treatment options, and clinical trials and then write reports in clear, concise, and plain English.

Candidates should have a Ph.D., preferably in a field of clinical science. An M.D. is not required but is favored.

Please e-mail or fax your curriculum vitae/resume to:

Benjamin Yang, M.D., Ph.D., Director of Research
PinnacleCare
250 West Pratt Street, 11th Floor
Baltimore, MD 21201
E-mail: resumes@pcistaff.com
Fax: 410-637-3431
Website: http://www.pinnaclecare.com

GRANTS

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Available in the United States and Canada
Application Deadline: March 16, 2006

The Brain Tumor Society (BTS) is awarding grants to fund basic scientific and translational research directed at finding a cure for brain tumors. Grants are awarded annually at a maximum of $100,000 per year. Grants may be used for startup projects or supplementary funding. Funds cannot be used for indirect costs. Clinical projects will not be funded.

For application packets, visit BTS **website: http://www.tbts.org.**

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